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MANUAL
FOR THE
PHYSIOLOGICAL LABORATORY

HARRIS & POWER.

MANUAL FOR THE PHYSIOLOGICAL LABORATORY.

MANUAL FOR THE PHYSIOLOGICAL LABORATORY.

BY

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THIRD EDITION.

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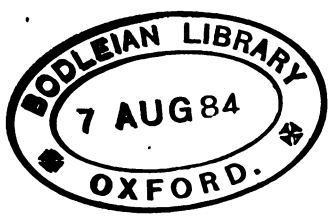

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PREFACE TO THE SECOND EDITION.

THE rapid sale of the first edition of this book, and the kind reception which has been accorded to it by many of the teachers of Physiology, both in the United Kingdom and in America, have encouraged the authors to bring forward a new edition.

Material alterations and additions have been made, and although the general arrangement has been maintained, it will be found that the sections on the methods of preparing tissues for examination have been almost entirely rewritten and corrected to date. In every case an endeavour has been made to describe those methods which are most practically useful, whilst many others have been purposely omitted as unnecessarily complicated. Various additions to the Histology, and the removal of some clerical errors, have rendered the second portion of the book more complete.

The section on Physiological Chemistry has been simplified by the introduction of more ample directions for the guidance of the student.

The third part comprises an account of some of the more simple physiological instruments, and of the chief experiments designed to demonstrate the properties of muscle and nerve. A considerable portion of this account has been used for some time in manuscript form in the authors' class, and has been found of service.

With an idea of impressing upon the student of medicine, for whom this book is more especially intended, the absolute necessity of acquiring a knowledge of healthy organs before proceeding to the examination of those which are diseased, a brief account of the ophthalmoscope, laryngoscope, and stethoscope has been appended.

In conclusion, the authors beg to offer their warmest thanks to Dr. Klein, F.R.S., and to Mr. F. J. M. Page, B.Sc., for much assistance in the sections upon Histology and Practical Physiology respectively. They have aided them materially, not only in suggestions and methods, but also in actual revision. The authors have also to thank Mr. Henry Power, Dr. Pye Smith, Dr. Lauder Brunton, F.R.S., Mr. McArthy, Prof. Charles, Mr. Butlin, Dr. Sawtell, Dr. Ormerod and Dr. Murrell, as well as those gentlemen who are mentioned in the preface to the previous edition, for suggestions and help.

By the courtesy of several firms, many figures have been inserted, in addition to those which have been specially drawn by Mr. T. Godart.

May, 1882.

PREFACE TO THE THIRD EDITION.

A THIRD edition of this Manual having been called for so soon after the publication of the second, the authors have only considered it necessary to correct minor details and to make small additions. With these exceptions, therefore, the present edition will be found to be identical with the previous one. The authors wish it to be understood that the omission of the authority for many of the statements in each part of the book has arisen from their desire to remove a difficulty from the path of the students for whom the manual was primarily intended.

March, 1884.

PART I.

PRACTICAL HISTOLOGY.

Epitome of Apparatus necessary for this Work.

Microscope.
A pair of scissors.
A pair of fine forceps.
Two scalpels.
Needles mounted in handles.
Razor.
Glass slides and thin cover-glasses.
Watch-glasses (6).
Section lifters (2).
Labels.
Filter papers.
A box or cabinet for mounted specimens.

Reagents.—Those in most common use are—

Potassium bichromate 1% & 2% solution.
Ammonium bichromate 5% „
Ammonium chromate 5% „
Müller's fluid.
Chromic acid ½% solution.
Osmic acid, gold chloride, palladium chloride, and silver nitrate.
Methylated spirit.
Absolute alcohol.
Clove oil.
Hæmatoxylin solution.
Picrocarmin „
Carmine.
Anilin colours.
Eosin.
Sodæ bicarb., 1% solution.
Acetic acid, „
Saline solution, 0·75%.
Tannic and boracic acids.
Glacial acetic acid.
Sodium chloride.
Glycerin.
Dammar varnish.
Canada balsam.
Distilled water.

PART I.

HISTOLOGY.

THE science of Histology embraces a knowledge of the various tissues of plants and animals, and of the means by which they may be prepared for examination under the microscope.

In some instances, as in the case of cartilage, the fresh tissue is sufficiently firm and resistent to enable a skilful operator to make sections without further preparation; but generally it is soft, and requires to be hardened by the application of re-agents, before its structure can be displayed; whilst many of the more interesting and delicate, as well as characteristic features, can only be rendered apparent after it has been subjected to the action of one or of several staining agents. Considerable experience is required to determine the best method by which particular details can be demonstrated; and the skill and judgment of the operator are shown by the order in which he applies his reagents, the strength employed, the temperature at which they are kept, the time during which they are allowed to act, as well as by the advantage he takes of accidental circumstances.

Very frequently much information may also be gained by subjecting specimens from the same preparation to various methods of treatment.

Examination of Fresh Tissues.

Tissues and organs, when recently removed from the body, do not often present satisfactory results when examined with the microscope. In some cases, however, it is requisite to examine fresh specimens, in order to observe some peculiarity of structure unaltered by reagents, or to observe some action, such as the

movement of cilia, which speedily ceases after the removal of the tissue from the body. Under such circumstances the tissue is either cut into thin sections by means of a freezing microtome, or a small piece is snipped off with scissors, teazed with needles, and mounted in one of the following solutions, when sufficient of its structure can generally be made out.

Normal saline solution—a 0·75% solution of sodium chloride.

Blood serum.

Aqueous humour of the eye.

Iodised serum,—*i.e.*, serum or liquor amnii to which iodine has been added for the purpose of preserving it.

Blood or muscle may, however, be examined without the addition of any reagent; but in this case evaporation of the serum must be prevented by the employment of means to be presently described.

Examination of Hardened Tissues.

The consistence of the tissues of the body does not, as we have said, often permit sections to be made of sufficient delicacy to display their minute structure, without being previously subjected to some hardening process. The chief exceptions are cartilage, bone, skin, kidney, and liver—from all of which, after some practice, satisfactory sections may be made. The hardening of tissues may be accomplished either by freezing or by means of various hardening reagents. The process of freezing is applicable to microtomes alone, and will be described under that head. The action of the hardening reagent is probably due to the coagulation of albumen, to the withdrawal of water, or in some instances, perhaps, to combining with the albumen to form a harder compound—an action which has been compared to the process of tanning.

HARDENING REAGENTS.

Chromic acid and spirit mixture is the fluid recommended for general use, and can be employed almost universally. It is thus prepared: ℞ Chromic acid $\frac{1}{6}$% solution (*i.e.* containing 1 grm. in 600 cc. distilled water), 2 parts; methylated spirit, 1 part.

This formula may be modified by taking equal parts of 0·5% solution of chromic acid and of methylated spirit. The mixture produces its effects in about seven to ten days.

Chromic acid, without the addition of spirit, in ·25 to ·5% solutions, is a rapid hardening fluid. The tissue is sufficiently hard in a week for sections to be made; if, however, it remains in the mixture for a longer time, it tends to become brittle.

Potassium bichromate in solutions, the strengths of which vary from 1% to 5%: the best is the 2% solution, and this, if changed every four days, hardens tissues in a fortnight.

Ammonium chromate in solutions 2% to 5% is believed by some to be superior in many ways to the similar potassium salt.

Ammonium bichromate is specially recommended for hardening the brain and spinal cord, in solutions of 5% strength. It must be prepared fresh when wanted.

Müller's fluid is made by taking potassium bichromate, 2 grms., and sodium sulphate 1 grm., and dissolving them in 100 cc. of distilled water. When this reagent is employed, the process of hardening is slow, but efficient. The fluid has the advantage of being exceedingly penetrating, and so will harden satisfactorily larger pieces of tissue than other similar agents.

Methylated spirit is a hardening reagent in very common use, and may be employed with advantage in the preparation of salivary glands, stomach and intestine, etc.

Absolute alcohol of specific gravity 0·795 is the most rapid hardening fluid. It is not often used, on account of its expense, and because it is said to have a tendency to produce shrinking of the tissue hardened, which preparations of chromium have not. The pancreas is one organ, at any rate, which should be immersed in this fluid, in preference to any other, for the purpose of hardening.

Osmic acid, 0·1 to 1%. **Gold chloride,** ·25 to ·5%. **Palladium chloride,** ·25 to 5%.	Harden, and at the same time stain tissues (see p. 19).

DIRECTIONS FOR HARDENING.

Never use anything but fresh tissue. This direction implies that nearly all the material used is taken from the lower animals; and this should be the case, as it is seldom possible to

obtain specimens from the *post-mortem* room sufficiently fresh to give satisfactory results.

Cut the tissue into pieces with a sharp knife or razor. The size of the pieces will vary with the reagent used. When chromic acid is the hardening fluid, the pieces should be of the size of a hazel-nut. Larger pieces may be cut for hardening in alcohol or potassium bichromate, and still larger for Müller's fluid.

Do not wash with water; but if it be necessary to get rid of any foreign body, allow a small stream of 0·75% saline solution, or of dilute spirit, or of a weak solution of potassium bichromate, to run upon the tissue from a wash-bottle.

Place the cut pieces in a large excess of the hardening reagent in a stoppered bottle, and keep the bottle in a cool place.

Change the hardening reagent frequently—e.g., the chromic acid and spirit solution on the second, fourth, and seventh days.

In all cases, in a week to ten days *remove the specimens to spirit* to complete the hardening.

SOFTENING REAGENTS

are used for two purposes—either to facilitate the teazing of tissues into their elements, or in order that sections may be conveniently cut of tissues otherwise too hard for the knife. Under these circumstances small pieces of the given tissue should be allowed to soak in one or other of the following solutions:—

Potassium bichromate 2%.—This is particularly useful for the purpose of dissolving up the cementing material between the fibres of tendon.

Baryta solution, for a similar purpose.

Iodized serum.

Weak methylated spirit, for lymphatic glands, spleen, and testicle.

Chromic acid, 5%, to which a few drops of sulphuric and nitric acid have been added, for bone and teeth.

Hydrochloric acid, 2% to 3%, or a mixture of this with nitric acid, 1%, for bone, teeth, and cartilage.

Picric acid, saturated solution, for softening teeth. Other fluids may be used for the purpose.

SECTION CUTTING.

Having hardened the material, the next and most important point is to cut thin sections from it; for unless the sections be thin, no amount of after preparation will make them fit objects for microscopical investigation. The methods which have been proposed from time to time to effect this object are numerous, but they may be divided into two classes,—viz., (1) Methods of cutting by hand; (2) Methods of cutting with machines called microtomes.

(1.) **Section cutting by hand.**—In cutting a small piece of tissue it is customary to embed it in some other tissue, or in a wax mass of some kind. For the first purpose, the tissue which is to be cut is placed between two pieces of hard liver,* or material of similar consistence, and held tightly in place between the finger and thumb, and cut with a razor in the manner to be described below. Instead of the liver, pieces of turnip, carrot, or potato may occasionally be substituted.

The usual method, however, is to embed the specimen in a wax mass. This will be described below.

EMBEDDING MATERIALS

are wax masses of some kind, modified according to the weather and the material to be cut. The following are those most commonly employed:—

White wax and olive oil, equal parts; melted and well mixed. This mass may be varied in consistency by diminishing the olive oil used.

Paraffin and lard.—Take five parts by weight of solid paraffin, and one part by weight of hog's lard and of paraffin oil; melt at a gentle heat, and mix thoroughly.

Spermaceti and castor oil.—Take four parts of the spermaceti and one part of castor oil.

Cacao butter may be used alone or combined with paraffin, wax, and oil, or with spermaceti and paraffin.

To melt the wax mass.—The wax mass is melted in a small porcelain capsule provided with a handle, over a jet of a Bunsen's burner or spirit lamp. Care must be taken that the material is not burnt. It is usual in laboratories to place the capsule on a

* Liver in a state of amyloid disease is best for this purpose.

piece of fine iron gauze on a tripod, and to place a gas flame from a Bunsen's burner of the smallest size beneath it; a glass rod may be used as a stirrer.

To embed the specimen in wax mass for cutting.—A piece of stout paper is taken, six inches long and three broad. This is doubled into three longitudinal folds; after this from each end folds of two inches long are marked off. The paper is then opened out, and of the three longitudinal folds the middle one forms the bottom and the lateral ones the sides of the paper box. The ends are made from the middle part of the end folds. The ends of each flap are marked off into two

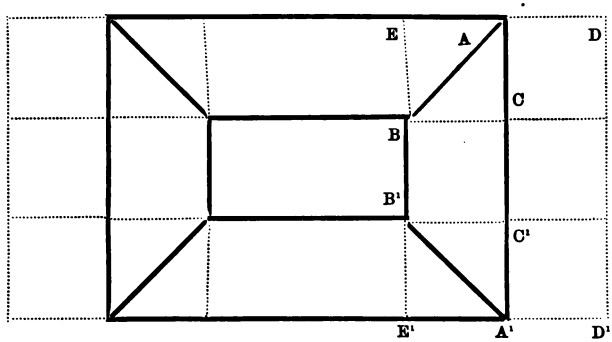

Fig. 1.—Diagram to show formation of embedding box.

equal squares, E C, C D, E^1 C^1, C^1 D^1. The squares E B A C and E^1 B^1 A^1 C^1 are doubled into two parts across the diameters

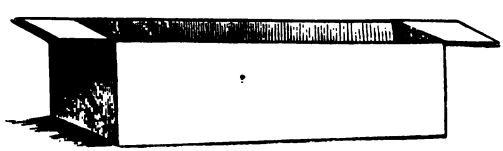

Fig. 2.—Paper box for embedding.

A B, A^1 B^1, and these triangular folds thus made are pinched up and pressed against the end of the box to support it; they are retained in position by the remainder of the end fold represented by A A^1 D D^1 being turned back over them.

Having made a paper box in the manner above described, and having melted the wax mass, take the specimen to be embedded upon a needle mounted in a holder, and having removed the superfluous absolute alcohol (in which the tissue ought to have been immersed for at least ten or fifteen minutes before the operation is commenced) with blotting paper, half fill the paper box with the melted wax mass, and dip the specimen into it several times, until it is thoroughly covered with thin layers of wax. Allow the wax to cool, and place the tissue on the wax in the box at one end; then fill the box with melted wax, and after it has hardened, mark on the outside the position of the tissue. When quite hard, turn out the wax and the embedded tissue by opening the ends of the box, and place for a few minutes in methylated spirit.

The tissue is now ready for cutting. It is as well to shave off the corners of the wax, and also to cut off several thin slices from the end near which the specimen is with a sharp knife, in order that when the cutting of the specimen is to be done, the razor may not be blunted by having to cut much wax.

Razors.—As a good deal of the success of hand cutting depends upon the sharpness of the razor, one cannot be too particular in choosing the instrument. It should be of good steel, capable of easy sharpening on a strop. It must also be broad bladed and hollow ground. The original "Army Razor"* answers all these requirements. It should always be stropped in one direction.

Section cutters, of various forms, have been introduced, but do not possess any advantage over razors.

DIRECTIONS FOR CUTTING SECTIONS BY HAND.

Be sure the razor is very sharp.

Hold the razor firmly in the right hand, with the fingers closed above the handle, take the wax mass between the index finger and thumb of the left hand, support the back of the razor on the former, and cut from left to right and from heel to tip of razor.

Let the handle be kept in line with the blade.

* To be obtained of Messrs. Arnold, West Smithfield.

Keep the blade well wetted with spirit, into which also the cut specimens must be floated off with a camel's-hair brush after each sweep of the razor; unless the specimen has been already stained and dehydrated, in which case clove oil is to be used instead of spirit for wetting the razor.

(2.) Section cutting with Microtomes.

MICROTOMES

are instruments which do away with the uncertainty of hand cutting. They are used when a large number of sections are required, and are invaluable for cutting for a large class. Some histologists prefer hand cutting for all delicate materials; indeed for some organs the microtome cannot be employed with satisfactory results.

The chief microtomes are—

Ranvier's microtome,* which consists of three hollow brass

Fig. 3.—Ranvier's Microtome, with glass top and winding screw (Beck).

cylinders fitting one inside the other; to the most external a flat circular brass plate is fixed at one end, and to the other a

* A modification of this microtome is made by Beck (see fig. 3). Drawings of the original microtome are to be seen in Schäfer's book and elsewhere.

cap which can be screwed on, carrying through its centre a fine screw. The tissue having been embedded in paraffin-wax in one of the cylinders, the cap is adjusted, the microtome held in the hand, and the screw turned until the wax is carried up sufficiently high for the embedded specimen to be cut; the flat plate guides the razor. After each section is cut the screw is turned slightly, thereby presenting sufficient of the specimen for another section.

Stirling's microtome is on the same principle as Ranvier's,

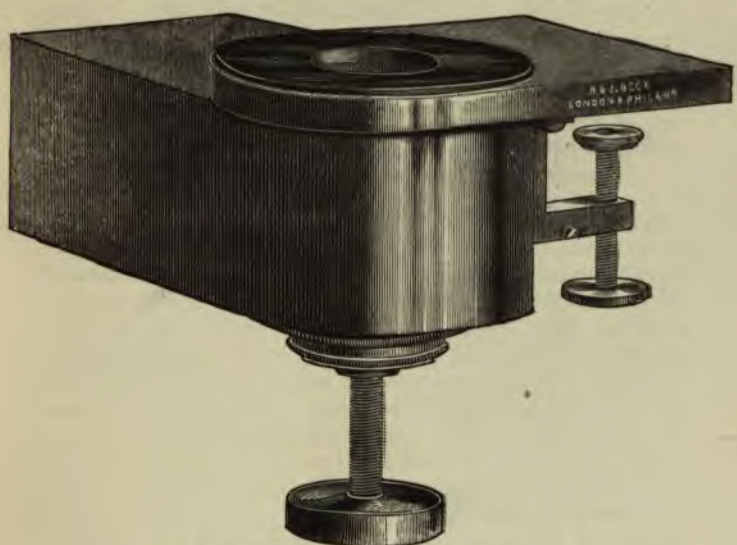

Fig. 4.—Rutherford's Microtome, with ice-box.

but is larger, and is fixed to a table by means of a screw. Only one cylinder, however, is fitted to the instrument.

Rutherford's microtome (fig. 4) provides a trough which may be used to contain a freezing mixture of ice and salt. When this is used, the tissue is embedded in thick gum, which, on freezing, becomes quite solid, and may be readily cut.

Both Stirling's and Rutherford's microtomes are generally provided with glass plates instead of brass, to guide the razor.

Rivet's is another form of microtome, in which the razor is arranged to slide at a fixed level (see fig. 5).

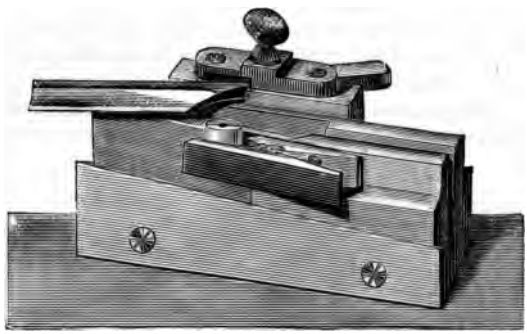

Fig. 5.—Section Cutter (Rivet's).

Roy's microtome * consists of a horseshoe-shaped piece of glass rod which serves as a guide to the razor, fixed by its two extremities into a brass plate. A bed is cut in the surface of this plate, along which a smaller plate in connection with a fine-threaded screw can be moved through small distances. The smaller plate is covered with a thin layer of cork, to which the specimen to be cut, embedded in white wax and olive oil, is attached. To the back of the larger brass plate a bent brass tube is fastened, which is intended to admit of a few drops or of a constant flow of spirit being projected on the knife and specimen whilst the sections are being cut. The tube is connected with a test tube arranged after the principle of a Wolff's bottle, and which can conveniently be suspended by a thread from the button-hole. A caoutchouc tube with a mouthpiece of glass attached to it permits of air being blown into the test tube, forcing out a part of the contained spirit or water through the bent brass tube.

Dr. Urban Pritchard's microtome.†—This instrument consists of a solid copper cylinder fitted with a wooden handle and a cap of thick felt. The copper cylinder has a diameter of

* An illustrated account of this instrument will be found in Foster's *Journal of Physiology*, vol. ii., p. 19.

† The *Lancet*, Dec. 11, 1875.

$1\frac{3}{8}$ inch and a length of $1\frac{3}{4}$ inch; the diameter of the end of the wooden handle is also $1\frac{3}{8}$ inch. The metallic cylinder is plunged face downwards in a mixture of finely pounded ice and salt: after remaining therein for three or four minutes it is taken out and wiped with a clean cloth. The instrument is thus cooled below freezing point; and if a piece of soft tissue be now placed upon its flat surface, it will immediately freeze and become adherent. The felt cap, which fits loosely around the

Fig. 6.– Williams' Freezing Microtome.

cylinder, should now be placed over the metal without touching the tissue, which will then rapidly become frozen throughout. The cap should now be reversed, so as to leave the metallic top free, and whilst the apparatus is held in the left hand, sections should be cut with the right by means of a razor which has been kept cool in ice and water.

Williams' microtome * is by far the best for general use.

* Made by Swift, Tottenham Court Road.

It consists of a circular wooden box about eight or nine inches in diameter. Into the centre is fixed a circular solid brass cylinder, with a brass plate at the top. This box contains a freezing mixture, and is provided with a waste tube to get rid of the water as the ice melts. The cover of the box is wooden, but has a plate of glass fixed upon its upper surface ; it also has a central aperture to admit the top of the brass cylinder. This cover is fixed down after the freezing mixture has been placed in the box, and fastened with a lateral screw. The specimen,

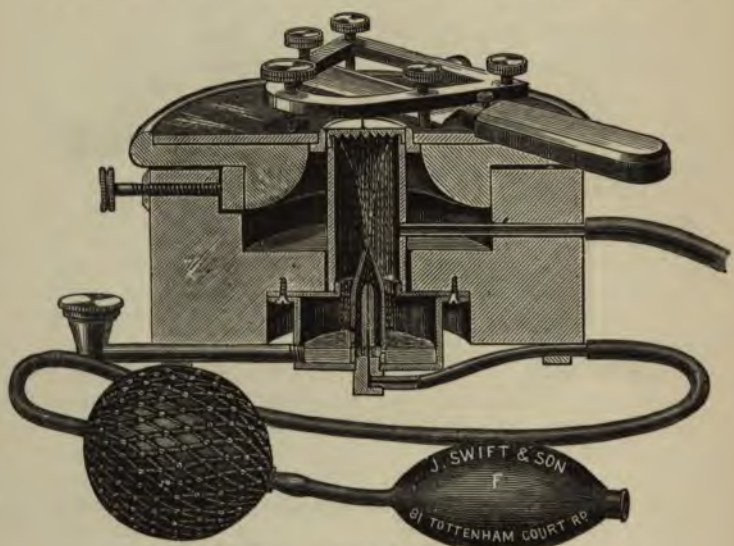

Fig. 7.—Groves' modification of Williams' Microtome.

which should first be left for twelve hours in water (to get rid of the spirit), is placed on the cylinder plate in a little gum. The gum is soon frozen, and the specimen is fixed by this means. The cutting is effected by means of a razor, which is fixed in a movable triangular brass frame, supported on three screws. By the adjustment of these screws the thinness of the section is regulated. The frame works smoothly over the glass table. The upper surface of the razor should be

slightly moistened with gum. As the sections are cut they should be swept off the razor with a camel's-hair brush, and should be washed in warm water before staining.

This microtome has recently been adapted for use, with ether as the freezing agent, by Mr. J. W. Groves (fig. 7).*

FREEZING.

Freezing mixtures are made by taking pounded ice, adding some rough bay-salt, and thoroughly incorporating the one with the other. In very cold weather snow may be obtained and used in place of the ice.

TO PREPARE GUM SOLUTION.

A strong solution of pure gum arabic in boiling distilled water is made, and filtered through calico. To this is added a few drops of melted pure carbolic acid. The proportion of gum is about a pound to a quart of water. It should be kept in a stoppered bottle, and boiled occasionally, to prevent decomposition.

TEAZING

is done with needles mounted in some kind of handle. These may be made very easily by taking a fine pointed needle, heating the eye red hot in a flame, and pushing it into a penholder or suitably cut piece of wood. More elaborate needle-holders may be bought of any maker of microscopic requisites.

Directions for Teazing.

Cut off a small portion of the tissue to be teazed with a sharp knife or pair of scissors, and place on a clean glass slide, in a small drop of the fluid in which the object is to be mounted (page 24)—generally glycerin. Place the slide upon a firm support as close to the eye as convenient, and with a couple of mounted needles separate the tissue into, as nearly as possible, parallel fibres. Some tissues cannot be so separated, and in that case they must be broken up with needles into minute pieces. Sometimes the aid of a lens or of a dissecting microscope (p. 35) has to be called in.

A rougher method than teazing may occasionally be used,

* *The Journal of the Quekett Microscopical Club*, vol vi., p. 293 (Oct. 1881).

when epithelium cells have to be examined. It consists of taking a scraping of the epithelial surface on a slide, adding a drop of glycerin, and gently tapping the cells with the smooth end of an ivory or bone penholder until they are seen under the microscope to be separate. This method is, however, almost certain to damage a certain proportion of the cells.

STAINING REAGENTS.

Almost all tissues require to be stained with some dye, in order that their structure may be more clearly shown. These dyes have the property of selecting and staining certain elements of the tissue, which would otherwise be indistinct. The following dyes are those which are usually employed :—

Hæmatoxylin or Logwood, in aqueous solution, is the simplest and best. It is made by taking *dried extract of hæmatoxylin*, 60 grms., alum in powder, 180 grms., and rubbing thoroughly together in a mortar, and adding slowly 300 cc. distilled water; mixing carefully and filtering; to the filtrate adding 20 cc. of absolute alcohol, and preserving in a stoppered bottle. The solution should be kept in a cool place for at least a fortnight before using. The older this solution, the more excellent it becomes.—A second but weaker solution may be made by adding a second 300 cc. of distilled water to the same logwood alum, and proceeding in a similar manner.

Hæmatoxylin, in alcoholic solution, is prepared by making saturated solutions of crystallized calcium chloride and of alum in proof spirit, mixing the solutions in the proportion of one to eight, and adding to this mixture a saturated solution of hæmatoxylin in absolute alcohol, drop by drop, until the whole becomes a dark purple. This solution may be used at once, but is greatly improved by keeping.

Carmine was formerly used much more than it is at present; it has the disadvantage of staining specimens a colour trying to the eye, whilst it has not the great selective power of hæmatoxylin. There are many ways of making carmine solutions, of which three are annexed.

(*a*) Take 2 grms. of carmine, and rub thoroughly in a mortar with a few drops of water; then add 4 cc. liq. ammoniæ and 48 cc. distilled water; filter into a bottle, which should be left unstoppered for a day or two for

the excess of ammonia to evaporate. This forms a strong solution, which must be diluted before using. (Klein.)

(*b*) Beale's solution is thus prepared: Dissolve carmine grm. i. in liq. ammoniæ fort. 3 cc. warm, add aq. destillat. 120 cc., and filter. Then add glycerini 30 cc., and spir. vini rectif. 120 cc., and keep in a well-stoppered bottle.

(*c*) Borax carmine is made by thoroughly mixing carmine (grms. ij) and borax (grms. viij) in a mortar, dissolving in warm water for twenty-four hours. The supernatant fluid, which should be decanted, is then ready for use.

Picrocarmin, *or solution of picrocarminate of ammonia*, is now much used, especially for "double staining" (p. 22)—*i.e.*, when it is expedient to stain tissues with two colouring materials, in order to bring out certain special features in their structure. It is prepared by adding a saturated ammoniacal solution of carmine to a saturated solution of picric acid until a precipitate forms, evaporating in a water bath to one-third its bulk—filtering and evaporating the filtrate to dryness: a crystallized mass, easily soluble in water, is obtained, which is picrocarmin. The strength of the solution should be about 1% to 3%. During preparation the ammonia should be kept in excess.

Another way is to take Beale's carmine without alcohol, and add picric acid in a similar manner. The glycerin prevents burning, which is not unlikely to occur.

Cochineal dye.—Take 7 grms. cochineal and 7 grms. alum in powder, thoroughly rub together in a mortar, and add 700 cc. distilled water; evaporate to 400 cc., filter twice, and afterwards add ʒj or ʒij absolute alcohol.

Eosin,* a fine rose-red dye, is used chiefly to stain nervous tissue and blood. It is very soluble in water, and requires to be fixed. In using this staining reagent, it is advisable to leave the tissue in a ·01% solution for twenty-four hours, and then to pass it through acidulated water. It is unnecessary to keep this substance in solution.

Aurin,† or corallin, is a reddish-yellow dye; it is used in alcoholic solutions.

* $C_{20}\,H_6\,Br_4\,O_5\,K_2$, a phenol dye.

† $C_{20}\,H_{14}\,O_3$, also a phenol dye.

Anilin dyes.—The following is a list of the chief anilin dyes, arranged in two classes, according as they are soluble in water or spirit respectively (H. Gibbes).*

(I.) *Soluble in water.*

China blue.
Soluble blue (Nicholson's).
Serge blue.
Tyrian blue.
Safranin.
Anilin black.
Dahlia, Rosanilin, and Methylanilin violet.
Malachite green.
Iodine green.
Bismark brown.

(II.) *Soluble in spirit.*

Spiller's purple.
Rosein (magenta).
Anilin scarlet.
Anilin violet.
Citranin.
Pure opal blue.
Anilin primrose.

In using anilin dyes it is necessary to stain specimens deeply, and to pass them through the desiccating and clearing fluids quickly, otherwise the colour passes into the solutions; for at present no one fixing fluid has been made which produces certain results; in some cases, however, a weak solution (about ·5%) of hydrochloric acid is effectual.

Of the anilin dyes the most useful are—

Soluble, or China blue.
Iodine green.
Methyl violet.
Anilin black.
Safranin.
Bismark brown.
} Soluble in water.

Spiller's purple.
Rosein.
Anilin violet.
} Soluble in spirit.

For these reagents to give satisfactory results, it is recommended to immerse the sections requiring to be stained for about twenty-four hours in the solution. This is especially applicable to iodine green, anilin black and Bismark brown.

* "Practical Histology and Pathology."

SILVER-NITRATE STAINING.

Silver Nitrate is used in all cases when it is required to demonstrate the flat tesselated or endothelial cells of serous membranes. This salt is taken up by the intercellular substance when fresh, and is reduced as a black precipitate under the action of light, and so maps out the cells in black lines. The fresh tissue should be placed after removal from the body in a ·5 or ·25% solution for ten or fifteen minutes; it should then be washed carefully in distilled water and exposed to the light in glycerin diluted with three times its bulk of distilled water. Silver nitrate is also used to stain nerve fibres.

Preparation of the solution of silver nitrate.—Powder 5 grms. of crystallized silver nitrate finely in a mortar, and add gradually 1000 cc. cold distilled water. After the salt has dissolved, preserve in a stoppered bottle of dark glass, or in one around which some black paper has been pasted, and keep in a dark cupboard. The use of the solid silver-nitrate in bringing into view the cell spaces of the cornea will be alluded to further on.

Ammonium-molybdate produces a bluish-grey general stain, which acts well as a base for double staining. A 5% solution in water may be used, and the specimens should be exposed to the light for twenty-four hours. The salt is expensive, and the advantage of its use not very marked.

Gold chloride selects and stains certain tissues, principally nervous; it also brings out the cells of fibrous connective tissue, cartilage and cornea.

Method of gold staining.—The tissue is removed from the animal immediately after it has been killed, and is placed in ·5% solution of gold chloride for half an hour to an hour; it should then be removed to distilled water for about twelve hours, and afterwards exposed to light in a saturated solution of tartaric acid until it sinks. Formic acid may be used in place of tartaric.

Method of preparing the solution.—The gold salt is sold in sealed glass tubes, containing about 1 grm.; the tube should be broken, and the salt should be dissolved and preserved in a manner similar to that described above under the heading of silver-nitrate staining.

Palladium chloride, in solutions varying from ·1 to ·5%,

is occasionally used; it has the same effect as gold chloride in hardening tissues and staining them at the same time. It is very expensive, and is seldom used.

Osmic acid, together with the two preceding salts, possesses the property of hardening as well as staining tissues placed in it. It is usually sold in 1% solutions, which may be diluted with distilled water at pleasure. The solution must be kept in a dark glass bottle. Osmic acid stains fat globules black, and *brings out* the medullary sheath of nerves. Specimens to be stained with this reagent must remain in it for about an hour, and should then be removed to spirit.

CLASSIFICATION OF STAINING REAGENTS.*

- *A.* General stains.
  - Carmine with excess of ammonia.
  - Eosin.
  - Ammonium molybdate. (This requires the action of light.)
- *B.* Selective stains.
  - Simple. Not requiring the action of light.
    - Carmine.
    - Borax carmine.
    - Logwood.
    - Logwood acid solution.
    - Indigo carmine.
    - Anilin blue.
    - Picric acid.
  - Simple. Requiring the action of light.
    - Gold chloride.
    - Silver nitrate.
    - Osmic acid.
    - Palladium chloride.
  - Double.
    - Ammonium molybdate and carmine.
    - Picrocarmin.
    - Palladium chloride and carmine.
    - Carmine and indigo carmine.
    - Logwood and anilin blue.
    - Gold chloride and logwood.
    - Silver nitrate and logwood.
    - Silver nitrate and gold chloride.
- *C.* Which will stain in the mass, and harden at the same time.
  - Osmic acid.
  - Picric acid.
  - Gold chloride.
  - Alcohol + borax carmine.
  - Alcohol + logwood.

* Groves'.

DIRECTIONS FOR HÆMATOXYLIN AND ORDINARY STAINING.

Such specimens as have been already selected as sufficiently thin should be thoroughly washed in distilled water or in methylated spirit, and placed in spirit in a watch-glass of suitable capacity.

Sections of tissues, hardened in any chromium preparation, must then be transferred to a watch-glass containing a solution of sodium bicarbonate 1%, and must be allowed to remain in it for five minutes, in order that the effect of the hardening reagent may be neutralized. They should then be well washed in warm distilled water (T. 30° to 40° C.).

To prepare the staining solution, it is as well to take a large watch-glass, fill it three-quarters full of distilled water, and then add to it four or five drops of aqueous hæmatoxylin. The hæmatoxylin solution is kept in a bottle provided with a funnel containing a filter paper, so that the reagent may be filtered before it is used.

Having made the solution, and thoroughly mixed it, place the sections in it carefully one by one with a broad needle, and if they float, press them down and leave them in the fluid for some minutes. The time which is required varies, as some tissues stain much quicker than others, and it is therefore necessary to take out a section from time to time, and to place it in a watch-glass full of distilled water, in order that the staining may be regulated. Great care is necessary, in order that the specimens be stained neither too much nor too little. It is recommended that during the examination of the specimen in the watch-glass it be looked at with a white background, which may be obtained by placing the glass on a white filter paper, or still better on a white glazed tile. This tile may easily be obtained, and will be found a great convenience.

It may not be out of place to notice here that stained specimens should always be examined during manipulation in this manner, and that unstained specimens can be most conveniently examined if placed upon a black plate.* This black plate is generally a

* Introduced, we believe, by Mr. George Walton, of St. Bartholomew's Hospital.

square piece of glass with the back blackened and protected with varnish. A dark-coloured glazed tile will answer the purpose.

When the sections are sufficiently stained, they must be washed in distilled water and placed in methylated spirit, ready for the next process (pp. 25, 26).

Watch-glasses and capsules of various sizes should be obtained.

Fig. 8.—Watch glass.

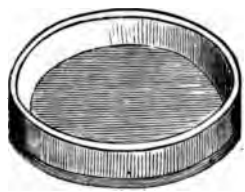

Fig. 9.—Glass capsule.

The small thin shallow glasses are of no use; those recommended are large, thick, and deep. The capsule which is used to contain spirit for hand cutting must be large and capacious—2½ to 3 inches in diameter, and 1½ inch deep.*

Directions for staining with eosin and anilin dyes.—Very similar precautions must be taken as in logwood or carmine staining, but, as has already been stated, specimens must, as a rule, remain in the solution for a much longer time (twenty-four hours or more), and the dye has to be fixed by passing the sections through acidulated water. There is no occasion for using sodium bicarbonate solution. When a spirit solution of the dye is used after staining, pass rapidly on to the next process. It is, however, exceedingly difficult to give any general directions for staining with these dyes, and the operator will soon find out the best methods of using each.

DOUBLE AND MULTIPLE STAINING.

Double staining.—To demonstrate the structure of some tissues satisfactorily, it is necessary to make use of two or more staining reagents. This process is called *double or multiple*

* To be obtained of Messrs. Orme & Co., Barbican, E.C.

staining, according as two or more dyes are used. The simplest form of double staining is that adopted for tissues which are first stained with silver nitrate, gold chloride, or similar reagent, and then with hæmatoxylin, carmine, or anilin. Other combinations may be made, of which the following are useful :—

Picrocarmin and hæmatoxylin.
Eosin and hæmatoxylin.
Eosin and anilin green, etc.
Picrocarmin and anilin, etc.
Anilin rose and anilin green.
Bismark brown and anilin green.

Multiple staining.—For a description of *treble* staining, the reader is referred to Dr. H. Gibbes' book.* It will suffice to mention here that the combination which he recommends is, (1) *picrocarmin;* (2) *rosein;* and (3) *iodine green.*

Staining with four colours† one of the authors finds may be done with good effect with (1) *picrocarmin or eosin;* (2) *logwood;* (3) *anilin rose;* and (4) *anilin green.* If the tissue has been already stained in gold chloride, five stains will have been used.

MOUNTING.

For this process the operator must have—

(1) *Glass slides*,‡ which are slips of glass three inches long and one inch broad, about the thickness of ordinary window glass, with or without ground edges. They are sold by the makers of microscopic requisites, at from 2*d.* to 6*d.* a dozen. It is as well to keep a good stock on hand; and, as a rule, within reasonable limits the thinnest are the best.

(2) *Cover glasses*,‡ which are made of extremely thin glass, circular or square, $\frac{1}{2}$ to $\frac{5}{8}$ inch in diameter. There are several kinds sold, usually known as ordinary, thin, and extra thin. The ordinary are quite thin enough for the student, but some-

* "Practical Histology," p. 39. 1880.

† "Multiple Staining," Stud. Journal, March, 1881; Report of Abernethian Society.

‡ These may be obtained of Tate, Holborn; Stanley, London Bridge; Beck, Cornhill; Medland, London Bridge; Baker, Holborn; Crouch, Barbican; and of many others.

times it is as well for him to provide himself with each kind. Dr. Gibbes* states that the ordinary cover glass measures from ·004 to ·008 inch in thickness.

To measure cover glasses.—Dr. Matthews† states that thin glass may be placed edgewise in the stage forceps of the microscope, and measured very accurately with the micrometer. The student may, however, neglect this operation.

To cut cover glasses.—Thin glass may be bought in sheets, and cut into squares or circles by the operator with a diamond, but this is not advised.

To clean slides and cover glasses.—For the former it is generally only necessary to wash them in soft water or weak soda water, drying with a clean cloth, and polishing with chamois leather. A mixture of ether and alcohol, caustic potash, infusion of nut-galls or alcohol, however, has to be used sometimes. Cover glasses may be washed in strong potash, or in infusion of nut-galls, or in alcohol, and should be dried and polished with a fine cambric pocket-handkerchief.

(3) *Section lifters*, which are easily made by beating out flat one end of a thick copper wire, four or five inches in length. The flattened portion should afterwards be filed at the edges, and rubbed smooth with sand-paper.

Fig. 10.—Section lifter.

More carefully finished section lifters may be obtained of instrument makers; and the annexed figure represents such lifters, suggested by the authors‡ for mounting large sections.

MOUNTING FLUIDS.

Fresh tissues may be mounted in any of the reagents which

* *Loc. cit.* The same author recommends a small steel gauge used for measuring sheet brass in fine work (to be obtained of Buck, Holborn) to measure cover glasses.

† "On Mounting Microscopic Objects." 1880.

‡ Made by Stanley, London Bridge.

have been mentioned (p. 4). The following fluids may also be used:—

Potassium acetate, a saturated solution is generally employed for mounting vegetable tissues.

Glycerin is one of the most useful of mounting fluids, and may be employed for fresh tissues, as well as for those which have been hardened and cut into sections. The fluid should be of high specific gravity; some microscopists advise dilution with a third of distilled water, but we cannot endorse this recommendation. It must be remembered that in this reagent some tissues swell up—*i.e.*, fresh tendons—and so lose their characteristic structure.

Directions for mounting in glycerin.—Place the tissue for a quarter of an hour in distilled water, transfer to the slide, spread out, and remove excess of water. Then place a drop of strong glycerin on a thin cover glass, and invert it over the specimen, taking care to keep the drop of glycerin in the centre of the cover, until the lowest point of the drop touches the centre of the specimen, and then allow the cover to fall gently on it. If the glycerin entirely fills the space under the cover, and is not in excess, the edges may be painted round with some cementing varnish.

Farrant's solution is a useful substitute for glycerin, to be employed for the mounting and preserving of sections and teazed specimens. It does not render the tissues so transparent as does glycerin; whilst the cover glass becomes fixed to the slide as the solution hardens. Farrant's solution is made by adding an equal weight of powdered gum arabic to a mixture of equal parts of glycerin and a saturated aqueous solution of arsenious acid.* The mixture is then allowed to stand for six weeks, being stirred at intervals. Any gum which remains undissolved is then filtered off; and the resulting clear filtrate is Farrant's solution.

Glycerin jelly is made by taking pure gelatin eight parts, soaking it in cold water for several hours, pouring off the water and warming the gelatin until melted, adding one part of egg

* The arsenious acid may be omitted, and a piece of camphor may be introduced in its place.

albumen, boiling until the albumen is coagulated and the gelatin is clear, filtering through flannel, and finally adding six parts of a mixture of one part of glycerin to two of camphor water. It is advisable to buy this reagent, as the making of it is difficult.

Carbolic acid solution (one in forty) may sometimes be used, or a mixture of this with alcohol and arsenious acid.

Castor oil is employed to mount crystals, etc., which are soluble in Canada balsam.

MOUNTING IN CANADA BALSAM AND SIMILAR REAGENTS.

When it is advisable to mount sections as permanent specimens, Canada balsam or a similar reagent is employed as the vehicle. There are, however, certain preparatory processes which have to be attended to before the sections can be thus mounted with satisfactory results.

Dehydration.—This process is effected by passing the specimens through methylated spirit and absolute alcohol. After staining they should remain in each of these fluids for five minutes.

Clearing.—After immersion in alcohol, the sections have to be placed in a fluid which will render them transparent. The fluid most extensively employed is *clove oil:* it is a hydrocarbon isomeric with oil of turpentine, and possesses the advantage over the latter of being more agreeable to manipulate. Oil of turpentine may, however, be used, as also some of the other oils which are isomeric with it. These fluids penetrate the tissues, and, as before mentioned, render them fit to be mounted in Canada balsam.

Summary of processes to be gone through before mounting in Canada balsam :—

It may be as well here to recapitulate the processes employed before mounting. If the section be cut in spirit or water, it is passed through—

Sodium bicarbon, 1%	If hardened in any preparation of chromium to neutralize the acid of the hardening reagent.
Distilled water.	To wash away excess of the colour.
Staining fluid.	

Methylated spirit. *Absolute alcohol.*	To dehydrate.
Clove oil, or turpentine. *Oil, benzol, etc.*	To render transparent.

Preparation of Canada balsam solution.—The best method is to take the commercial balsam, expose it to a temperature of 70° C. for twelve hours, to dry it and render it quite hard, and then dissolve in benzol and filter. It should be preserved in a stoppered bottle.

Another method is to mix equal parts of Canada balsam and chloroform, and warm. The balsam is entirely dissolved. Filter.

Preparation of Dammar solution.—Dissolve gum dammar in powder 50 grms. in 150 cc. turpentine, and filter; gum mastic 50 grms. in 200 cc. chloroform, and filter. Mix the solutions, and again filter. This solution can be employed in place of Canada balsam.

Drop bottles for Canada balsam, Dammar, etc.—Mounting fluids such as Canada balsam are usually kept in bottles with

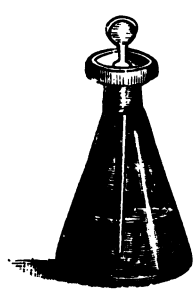

Fig. 11.—Drop bottle for Canada balsam, etc.

narrow elongations of the stopper, which dip into the fluid, and will deposit it on the cover glass in drops (fig. 11).

Directions for mounting in Canada balsam and Dammar varnish.

Having placed the sections in clove oil in a watch-glass or suitable glass dish upon a white plate, as above directed, take a

clean slide (called also the object glass) and place it upon a white filter paper. Next, by means of a section lifter and a needle, withdraw a section from the clove oil, and bring it down upon the centre of the slide, and remove the excess of clove oil with filter paper. Then take a clean cover glass, and place a drop of the Canada balsam solution on the centre of it, and proceed in the manner described under the head of Glycerin Mounting (p. 25), or take hold of a corner with a pair of forceps, and gradually incline it over the specimen, allowing the opposite edge to touch the slide first outside the specimen, and then allowing the other part held with the forceps to fall gently on the specimen. Either of these methods will prevent the formation of many air-bubbles under the cover glass. It is almost impossible not to have some, but these will disappear if the specimen be kept in a fairly warm place for a few hours.

CEMENTING REAGENTS.

Sections mounted in glycerin and in similar fluids which have been mentioned above must have, and those mounted in Canada balsam or Dammar varnish may have, their cover glasses secured with cementing material which is painted on with a camel's-hair brush. It would be useless to mention more than a few of these cementing fluids. The most useful are—

Dammar varnish, prepared according to the above formula.

Canada balsam, made with benzol.

Brunswick black, which is asphalte dissolved in turpentine or naphtha.

Gold-size.

Marine glue (*Hollis').—The authors find this the most convenient cement, as it dries very quickly.

ACCESSORY APPARATUS.

Boxes and Cabinets.—Having prepared and mounted as permanent specimens any tissues, it is necessary to label them carefully, noting the method of preparation, the mounting material and

* To be obtained of Baker, Holborn.

date, and to set them aside in a tray box, such as is seen in the figure; these may be obtained of the instrument makers. The

Fig. 12.—Tray box for mounted specimens.

boxes are arranged to hold from 1 to 6 dozen slides; when a larger collection has been made, the worker will find a cabinet very convenient.

Fig. 13.—Cabinet for mounted specimens.

Knives, Scissors, Forceps and Needles.

Knives.—For the various operations of dissecting tissues, cutting in pieces and scraping, ordinary scalpels are very useful.

For more delicate dissections, etc., other knives are used,—*e.g.*, those principally employed by ophthalmic surgeons, and figured below (fig. 14).

It is hardly necessary to add that knives must always be kept very sharp.

Fig. 14.

Scissors.—Must be fine and sharp, and of several sizes when possible; the diagram below will indicate several kinds.

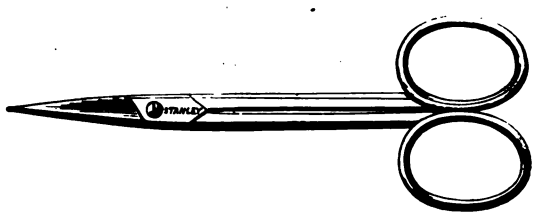

Fig. 15.

Forceps.—Should be fine-pointed and slender, if choice be

Fig. 16.

allowed, but dissecting forceps are quite suitable for ordinary work.

Needles.—As before mentioned, a sewing needle inserted into a piece of wood, leaving about $\frac{1}{8}$ or $\frac{1}{4}$ inch of the pointed end uncovered, is quite sufficient for all purposes. Broad needles are also very useful.

*Cabinets for microscopic mounting** have been arranged by several instrument-makers containing all the requisites for the student in a compact compass. The prices vary from £1 1*s.* to £1 7*s.* 6*d.* They save the beginner some trouble.

Fig. 17.

THE MICROSCOPE.

Description of Microscope. *A compound microscope.*—In describing a microscope for the use of those commencing the

* The best we have seen are made by Stanley, London Bridge, and Medland, High Street, Borough.

subject of Histology we select the small so-called *continental* model, of Hartnack, Nachet, and others, as being at once the most durable and the most simple of the various forms of instrument in common use.* It consists of a lacquered brass stand carrying a hollow cylinder in which the tube of the microscope slides. At

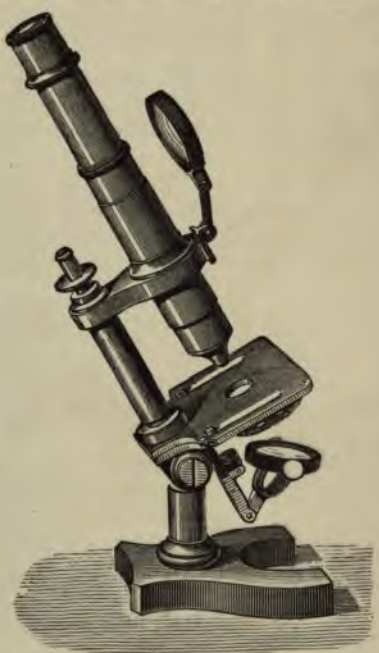

Fig. 18.—The Continental model microscope.

the top of the stand is a screw with a milled head which works "the fine adjustment"; when the screw is turned, the tube of the microscope is moved very slightly to or from the stage. In the

* The above illustration of one of M. Nachet's microscopes is almost exactly similar, except in details, to some of the models of Messrs. Hartnack and Prazmowski, M. Verick, and Zeiss. And fig. 19, which is a drawing of Crouch's £5 5*s*. microscope, is very similar to those of other English makers (see p. 39).

simpler forms the "coarse adjustment," or the freer movements of the microscope tube up and down, is effected by the hand, whilst in the more expensive models it is performed by a rack and pinion. The "stage" of the microscope, upon which the glass slide is placed, is a flat plate of brass blackened upon its upper surface, and perforated by an aperture in the centre. Through the aperture light is reflected from a mirror; the

Fig. 19.—English model.

amount of light admitted to the lens is regulated by a small cylinder which fits into the central hole; withdraw this from below: it will receive at its upper end one of the three brass stops or caps which are found in the shagreen lens case. These stops are the diaphragms; they are perforated by apertures of different sizes; the smallest is a mere pinhole, and is for use with the

strongest magnifying power. Insert one of the diaphragms into the cylinder, which should then be replaced in the centre of the stage. When in position it is exactly flush with the upper surface. In some microscopes this form of diaphragm is replaced by a blackened ring of metal perforated near its circumference by holes of various sizes, and revolving round its centre. At the back of the stage is a pair of brass clips for holding the slides in position when the microscope is tilted. In more complicated instruments the movements of the stage in different directions are effected by screws; whilst in a Hartnack the stage is fixed, and the finger and thumb suffice to move the slide upon it.

Beneath the stage is a movable mirror with two faces, one concave, the other plane: the concave mirror is the more commonly used. The tube of the microscope consists of a hollow brass cylinder about five inches in length. It contains a second tube, which can, if necessary, be drawn out, in order to increase the magnifying power. The upper extremity of the tube receives the "ocular" or eyepiece: the oculars vary in magnifying power, the one of lowest strength being numbered, in Hartnack's system, 2, that for common use 3, and the higher 4, 5, etc. It is better not to change the oculars, but always to work with the same—*e.g.*, No. 3. Into the lower end of the tube the "objectives," "powers," or "lenses" should be screwed. Of these the lowest is No. 4, the highest No. 9: No. 4 focusses about $\frac{1}{2}$ an inch from the object; No. 8 about $\frac{1}{8}$ of an inch. In using the microscope, put on the low power, No. 4, *first;* then adjust the mirror in such a way as to get a full illumination of "the field"—*i.e.*, when the eye is applied to the objective to see a circle of light of equal intensity in all its parts. Never use the direct rays of the sun. Next put the object to be examined upon the stage in such a way that the section is over the centre of the aperture: the stop with the largest hole is to be used. Bring the lens to within half an inch of the slide, and carefully employ the fine adjustment until the object is distinctly seen; then alter the focus so as to observe it at various depths. After examining with the lower power, remove it and replace by No. 7 or No. 8, at the same time changing the stop for a smaller one: bring the lens down until it almost touches the object, and then focus by

means of the fine adjustment. Students are apt to leave a portion of the lens attached to the cylinder when they change the powers, and so to screw a No. 8 on to half a No. 4; the field is often obscured by drops of glycerin, Canada balsam, etc., adhering to the bottom of the lens. Attention may be directed to these points when it is found that the object cannot be focussed as usual. A drop of methylated spirit upon a soft handkerchief is sufficient to remove Canada balsam from lenses. A "condenser," or lens fitted into a jointed lever which slides up and down the tube of the microscope, is provided: it is employed for condensing light upon opaque specimens; it may, however, be removed, since it is scarcely ever needed in the modern histology of medical students, as the majority of specimens are prepared for examination by reflected light.

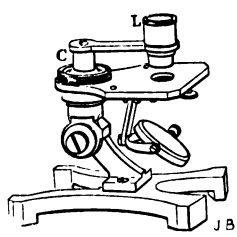

Fig. 20.—Nachet's microscope as a dissecting microscope.

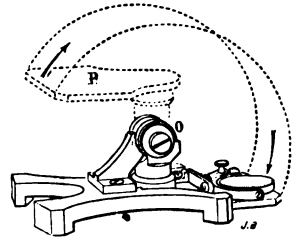

Fig. 21.—Method of packing Nachet's microscope.

The simple or dissecting microscope (fig. 20) consists essentially in a stage illuminated from below by a movable mirror. Above the stage is an arm which can be raised or lowered by a rack and pinion (C). The arm carries a lens (L) similar to that employed by watchmakers. M. Nachet, of Paris, has invented a model* in which the ordinary compound microscope can readily be replaced by the dissecting microscope (fig. 20). This is effected by simply unscrewing the tube of the compound microscope from the arm which carries it, and screwing in a simple lens. The dissecting microscope is of use in certain cases where it is necessary to teaze or otherwise manipulate very delicate tissues.

Lenses or Objectives.—For ordinary use the student re-

* Microscope portatif de voyage.

quires two objectives: one of long focal length, which magnifies about 60 to 100 times; and another of very short focal length, which magnifies 300 or 400 times. Objectives such as these are known by different numbers or letters, according to the maker—*e.g.*, English makers would call them 1 inch and $\frac{1}{4}$ or $\frac{1}{8}$ inch objectives, and Hartnack (Paris) 4 and 7, and Zeiss (Jena) A and D. Other lenses named on the same system may be obtained

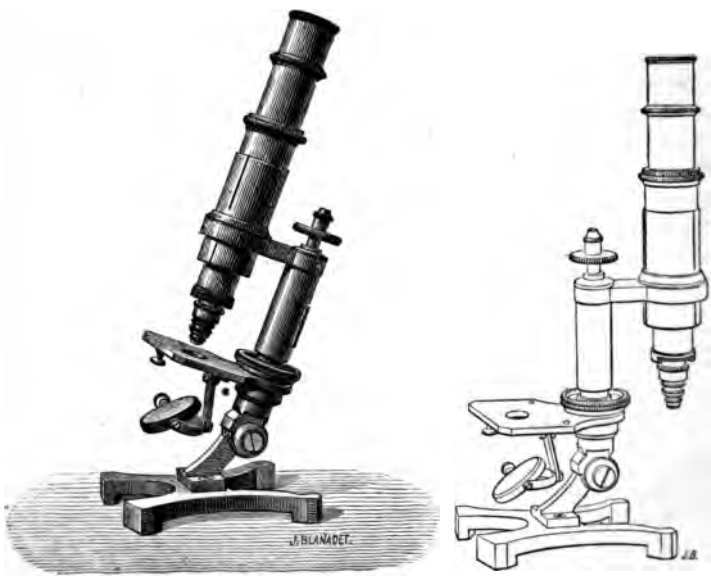

Fig. 22.—Microscope portatif de voyage.

when necessary. We have no hesitation in strongly recommending Zeiss' lenses as the very best we have tried; the A and D are sufficient for general work, but the higher objectives, especially some of the immersion lenses, are also excellent. Hartnack's objectives are held in general repute, but do not seem quite so certain as formerly. The objectives of Beck, Crouch, Ross, Swift, Powell and Leland, among English glasses,

and of Nachet and Verick among foreign glasses, are also, as a rule, very satisfactory.*

Immersion Lenses.—When very high powers are used, it has been found best to fill the interval between the lens and the cover glass with some fluid, to diminish the index of refraction. Cedar-wood oil is now usually employed. The mounted specimen to be examined must be sealed round with marine glue, and a drop of the oil must then be placed upon the centre of the cover glass with a glass rod, and the lens brought down to the oil and focussed carefully. A better illumination and definition is thus obtained. Students do not, however, often need to use immersion lenses.

Oculars, or eyepieces, are named or numbered in a manner somewhat similar to their objectives; but as a rule are known as 2, 3, 4, etc. The student is advised, as before mentioned, not to use a high ocular. In Hartnack's system, No. 3 is the one usually employed; any higher than this is not perfectly achromatic.

Light.—The best light for microscopic work is that afforded by the sun, when its rays are not too powerful. A fine sunny spring day affords what may be called a typically favourable light. The light should be considered best when reflected from white clouds in a blue sky. In England, however, we have only too frequently to make use of artificial light. Various forms of gas and oil lamps have been suggested. We annex one form in fig. 23. At St. Bartholomew's we use gas argand burners with blue glass funnels.

Fig. 23.—Lamp for work with microscope.

Of drawing microscopical objects.—If the student is a good draughtsman, practice is the only thing required before he can make good drawings of microscopic

* It may perhaps seem to be invidious to mention the names of certain makers to the exclusion of others; but in a practical book as this aims to be, the authors feel obliged to give the result of their own experience, and possibly omit the names of many excellent makers of whose work they may be only partially cognisant.

tissues. Many others will very possibly bring out latent talents if they will practise. One of the greatest histologists of the day informed the authors that he had been driven to cultivate the art of drawing simply because the drawings of others did not seem to adequately represent his specimens; he now is a first-rate draughtsman. Some, however, whatever their perseverance, will need aid; and this is given them by an instrument called the *camera lucida*.* This consists of prisms arranged in such a manner that, when attached to the microscope tube by a holder, and placed exactly over the eyepiece, the image of the object is thrown upon some part of the table where a sheet of white paper may be laid, and the outline of the object can then be traced upon it. An instrument constructed for a similar purpose is Beale's neutral tint reflector, which is thus used: The cap of the eyepiece is removed, and the reflector is applied in place of it. The microscope should then be inclined to a horizontal position, and at ten inches from the table, and the paper is placed exactly underneath the reflector. After the object is focussed and properly illuminated, the eye should be brought *close to*, and *exactly over*, the reflector, and the image will then appear to be thrown upon the paper, and may readily be traced.

Fig. 24.—Neutral tint reflector (Beale's).

Directions for choosing a Microscope.—Great care is necessary in the choice of a microscope, and the student is recommended not to buy one without asking the advice of some one who is well acquainted with modern instruments. We may, however, tell him roughly what not to get. Let him not buy one of those large constructions of brass which are so often strongly recommended by dealers, or one which has a complicated arrangement of screws and buttons to move the object glass. It would be simply paying money for useless material. We cannot recommend binocular microscopes for students' use, or those in which the fine adjustment tilts the draw tube forward. Second-hand instruments, except modern instruments

* Zeiss' is a good form of camera.

of well-known makers, are to be avoided, as are also old instruments, of whatever kind. The following points of advice may be of some use:—

The stand must be small, and at the same time firm; the fine adjustment delicate and stedfast, and the lenses and oculars must be clear and perfectly achromatic.

The question which must be considered first of all in the choice of a microscope is, how much money is proposed to be given for the instrument; if £5 5*s.* or under, the student cannot do better than purchase an English microscope of a maker, of whom we may mention *R. & J. Beck*, 68, Cornhill; *H. Crouch*, 66, Barbican, E.C.; *J. Parkes & Son*, 5, S. Mary's Row, Birmingham; *James Swift*, 81, Tottenham Court Road. All of these makers charge about £5 5*s.* for each microscope. Messrs. Parkes also make a fair instrument for rather under £4. The cheap microscopes of *Ernst Leitz* (Wetzlar), £5 5*s.* and £3 10*s.*, may also be mentioned. If the student is prepared to pay more than £5 5*s.*, we recommend a microscope constructed by *Hartnack & Prazmowski*,* 1, Rue Bonaparte, Paris, (who will send a catalogue on application.) Stand. VIII., with oculars Nos. 2 and 3, and objectives 4, 7, and 8, is an excellent form, and costs about 250 frcs. More expensive stands and higher objectives may be also obtained from the same firm, when the student has mastered the rudiments. *Zeiss*† (*Jena*) makes first-rate stands, and, as we have before said, *the best lenses*. The stands are rather expensive. Stativ V. b., with objectives A and D, and No. 3 ocular, costs about £9; and Stativ VIII., with objectives A and D, with 1 ocular, costs about £6 5*s.* Zeiss's stands possess the advantage that they will take English lenses, and *vice versa*. This is not the case with Hartnack's.

For a thoroughly complete microscope, we believe there is no better than Zeiss's No. 1, the price of which, together with his lenses, is practically unlimited. The stand alone costs about £15.

Nachet‡ (17, Rue Saint Severin, Paris). No. 9, with objec-

* Messrs. Hartnack's microscopes are kept in stock by Marr, 27, Little Queen Street, Lincoln's Inn, W.C.

† Messrs. Baker, 22, High Holborn, are agents for Zeiss, and supply his microscopes at catalogue prices.

‡ Agent—Medland, 54, Boro' High Street, S.E.

tives 3 and 6, and two oculars, is an excellent instrument, and costs £6 10*s*. We are very pleased with his No. 18, *microscope portatif de voyaye* (fig. 22), the cost of which is £8.

Verick's (2, Rue de la Parchemenaire, Paris) microscopes are good, but expensive.

As regards the higher class microscopes of English makers, we cannot help thinking that the prices are too high, and that there is a tendency to make the instrument cumbersome. We can, however, with justice mention with praise some instruments made by each of the makers mentioned above.

A nosepiece (fig. 25) is employed for the purpose of readily changing one objective for another. It is especially useful when it is necessary to examine an object with a high and low power alternately. Nosepieces are constructed to carry two, three, or

Fig. 25.—Nosepiece.

four objectives. The one represented is for two powers. It is attached to the tube of the microscope by a screw; one objective is screwed on at B and a second at C; by reversing the position of B and C, a change of power is readily obtained.

INJECTION.

Injection Materials.—The injection material should first be prepared. It consists of a mixture of carmine and gelatin, made as follows:—Suspend 4 grams of carmine in a minimum of

water, adding 8 cc. of liq. ammoniæ, and afterwards 48 cc. of distilled water; filter the solution. Dissolve 13 grams of the best gelatin in 100 cc. of water by the aid of heat derived from a water bath, and filter. Add the carmine solution to 72 cc. of the warmed gelatin filtrate. Then add, with constant stirring, 4 or 5 cc. of glacial acetic acid to each 18 cc. of the carmine and gelatin solution, kept at a temperature of 40° C.

A solution of gelatin prepared as above, with the substitution of Berlin blue for the carmine, forms an equally good injecting fluid. The Berlin blue is an aqueous solution of a strength of 2%, made by dissolving 10 grams of Brücke's soluble blue in 500 cc. of distilled water.

Methods.—The animal to be injected should be killed by chloroform, in order that the arteries may be dilated to their utmost extent, and an incision into one of the larger blood-vessels should then be made. Whilst it is still warm it should be immersed in a bath of water at a temperature of 40° C. Tie a nozzle provided with a stopcock into the vessel previously opened—carotid, femoral, or crural, as the case may be—fill the nozzle with saline solution by means of a pipette. Then attach it to the syringe (fig. 26), previously filled with the injection material, which has been rendered fluid by warming it, and push down the piston so as to drive the injection material into the vessel. This must be done very slowly indeed, and the progress of the injection should be ascertained from time to time by examining the more vascular organs of the body; *e.g.*, the tongue or ear.

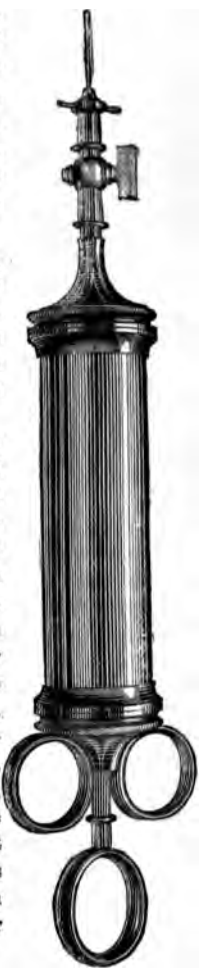

Fig. 26.—Injecting syringe.

The injection of particular organs, such as the kidney, is effected by inserting the nozzle of the syringe into the main artery leading to it; great

care and long experience are, however, necessary to obtain successful results.*

The lymphatics can readily be demonstrated in the intestine by injecting them with a watery solution of Berlin blue. This is done by inserting the point of an ordinary hypodermic syringe, filled with the staining solution, into the coats of the intestine in the neighbourhood of a Peyer's patch, and then gently pressing down the piston.

After injection in the case of the entire animal, the body should be transferred to a large vessel, containing ice-cold methylated spirit, without removing the nozzle from the artery. When isolated organs have been injected, they should be suspended in ordinary alcohol in a beaker.

THE BLOOD.

HUMAN BLOOD.

Prick the finger with a needle, and apply a clean cover glass to the drop of blood which issues, so that a small quantity is deposited in the centre; then mount it on a perfectly clean glass slide, and examine with a magnifying power of 200-300 (Hartnack ocular 3, objective vii.). Notice that there are various kinds of corpuscles contained in a fluid menstruum (serum).

A. **Coloured Corpuscles.**—Circular discs $\frac{1}{3000}$ to $\frac{1}{4000}$ in. in diameter, and $\frac{1}{10000}$ in. in thickness, depressed a little on each side. When seen sideways, biconcave or dumb-bell shaped; of a pale buff colour; but when aggregated, of a reddish tint. They have a tendency to run together, collecting in rolls or rouleaux. Notice a corpuscle as it rolls over, and observe the change in its form, that it is alternately circular and biconcave.

* Fuller details of the various methods and the different apparatus employed in the process of injection will be found in the "Handbook for the Physiological Laboratory," pp. 97—103 (by Dr. Klein), and Schäfer's "Practical Histology," pp. 142—150, and pp. 200 *et seq.* Students are not often required to perform this operation.

The corpuscle has no nucleus; the false appearance of a nucleus is occasioned by the refraction of light in passing through a biconcave disc. Prove this by slowly altering the focus. The centre of the corpuscle is seen to become lighter than the periphery when out of focus. The corpuscles consist of two parts: a stroma, which is colourless, and the coloured part, a red crystallizable substance, *hæmoglobin*.

B. **Colourless Corpuscles.**—Their proportion to the coloured varies from 2 to 10 in 1000.

When perfectly fresh, they are spherical and faintly granular; they quickly alter, and become markedly granular.

About $\frac{1}{3500}$ inch in diameter nucleated, the nucleus not often apparent without the addition of weak acetic acid. They are nearly always isolated; do not collect together or mix with the coloured discs. They are endowed with the power of spontaneous motion (amœboid movement). To demonstrate this property it is advisable to make use of a warm stage. (See Part III.)

Action of Reagents on the Blood: Irrigation.—Take a small drop of blood on a slide, and add to it a drop of saline solution. Cover it with a glass cover; any reagent may now be made to act upon the blood by placing a drop of it on one side of the slip, and applying a piece of filter paper to the opposite side. A stream of the fluid passes under the cover glass. This is called *Irrigation*. Irrigate specimens of blood with—

(1) *Water:* the red corpuscles become smooth and pale, and disappear.

(2) *Acetic acid:* the same changes take place; the colourless corpuscles become more distinct, and their nuclei more apparent.

(3) *Tannic acid:* the hæmoglobin collects in small lumps at the sides of the red corpuscles.

° *Gases.*—A gas chamber may be made by taking a slide and placing upon it a circle of putty; beneath the putty two small glass tubes are thrust, so that their ends extend into the cell thus formed. The blood is placed upon a cover glass which is inverted upon the ring of putty. One of the glass tubes is then connected by means of an india-rubber tube with the bottle in which the gas is generated. The stream of gas is

The sections marked o may be omitted by the junior student.

allowed to pass into the chamber, and its action upon the blood corpuscles is observed.* The gas passes out of the chamber through the second tube.

° *Action of Carbonic Acid Gas.*—The preparation is brought into focus, and the gas is allowed to pass through the chamber. *Human.*—The red blood corpuscles, which had become crenate from the action of the salt solution, again acquire a smooth outline, owing to the swelling up of the parts between the projections. On admitting air to the chamber the corpuscles again become crenate. The nuclei in the white corpuscles become more distinct. *Amphibia.*—The nuclei in the red corpuscles appear more distinct, owing to the coagulation of the substance surrounding the nucleus.

° *Action of Chloroform on the Blood.*—Preparation as for gases. The red corpuscles become globular, the hæmoglobin being finally dissolved and discharged into the plasma; the blood, when seen with the naked eye, being transparent (lake) and no longer opaque.

° *Action of Electricity on the Blood.*—The blood is placed on a slide in such a position that when it is covered it spreads between two poles of tinfoil situated six millimeters apart, which are connected with the secondary coil of an induction apparatus. After a succession of *induction* shocks the *red corpuscles* lose their smooth outline, become crenate, then like mulberries, and finally horsechestnut-shaped. They swell up, and ultimately become decolorized. The *white corpuscles* swell up and disintegrate, their granules exhibiting molecular movements. With a *constant current* from a single Bunsen's cell the *red* corpuscles at the positive pole undergo changes which correspond to those exhibited under the action of an alkali, and at the negative pole of an acid. The *colourless* corpuscles assume a spheroidal form, the amœboid movements being resumed as soon as the stimulus has passed.

The Action of Alkalies on Blood.—A mixture of 2 cc. of caustic potash in 1000cc. of saline solution causes both red and white blood corpuscles to swell up, burst, and disappear. The red corpuscles appear to be more rapidly affected than the white.

The Action of Alcohol.—A mixture of one-third spirit and

* For description of the warm stage see Part III.

two-thirds water, acting upon amphibian blood, causes the nucleus to swell, and brings into view the nucleolus in the red corpuscles. It also renders the nuclei of the colourless corpuscles more evident, whilst one or more delicate and clear "blebs" grow from the periphery of the white corpuscle. These "blebs" appear to consist of some colloid substance, into which endosmosis rapidly takes place.

° *The method of Feeding the Colourless Corpuscles.*—The preparation of blood on the warm stage is irrigated with carmine, vermilion, or anilin blue, in a finely divided state (for method of preparation see "Handbook for the Physiological Laboratory," p. 10), or with milk. The white corpuscles will be found after a short time to have ingested some of the finer particles.

The particles are taken into the substance of the corpuscle by the union around it of two of the protoplasmic processes, and they thus lie at first close to the periphery of the cell, being carried at a later period nearer to its centre.

° *Action of Carbolic Acid on the Blood.*—With dilute solutions *the red* corpuscles shrink, and lose their regular contours; after a time they swell up, become pale, and disintegrate; *the white corpuscles* in a dilute solution of carbolic acid in saline solution (1-800 to 1-1600) do not continue their amœboid movements for so long a time or so rapidly as they otherwise would, and the movements are generally of simple extension and retraction of processes, no locomotion taking place. When the corpuscles cease to move, they do not disintegrate as rapidly as when the carbolic acid is absent. With stronger solutions the *red* corpuscles become granular, and the stroma breaks up to form a homogeneous brownish-red material of a high refractive index, which may appear either as an irregular network within the cell, or in the form of globules which tend to coalesce, and are insoluble in water, but are dissolved by carbolic acid. With solutions of $\frac{1}{50}$ to $\frac{1}{20}$, the movements of the white corpuscles cease instantly, the cells shrink, and become coarsely granular. (Prudden).

Hæmin Crystals.—A drop of blood is dried on a glass slide. Two or three granules of common salt are added. With a capillary pipette add a drop of glacial acetic acid, and then cover. Raise the temperature gradually to the boiling point over a spirit lamp, until the greater part of the acid has evapo-

rated. A number of small reddish-brown rhomboidal crystalline plates are seen.

° **Hæmoglobin Crystals.**—Take a drop of blood from a guinea-pig, and let it coagulate on a slide; add a little water, and take up the clot with the forceps, and let several small drops fall upon another slide. As these evaporate, hæmoglobin crystals of various sizes shoot out from the edges, separately and in bundles.

For the chemistry of the blood see Part II.

BLOOD CORPUSCLES OF OTHER VERTEBRATE ANIMALS.

Coloured Corpuscles.—In nearly all mammalia the coloured corpuscles are round, disc-like, non-nucleated bodies, similar to those of man, but differing in size. In this respect they vary considerably.

In birds, reptiles, amphibia, and fishes, the corpuscles are oval and nucleated, the nucleus presenting a central elevation on each surface. These corpuscles are larger in birds than in mammalia; still larger in fishes; and of a yet greater size in amphibia.

Colourless Corpuscles.—The general characters of these corpuscles are similar in all animals, but are found in much larger proportion in the blood of fishes and amphibia than in that of mammalia and birds.

Irrigate a specimen of newt's blood with boracic acid 1%. The hæmoglobin collects around the nuclei of the red corpuscles.

EPITHELIUM.

SQUAMOUS EPITHELIUM.

(*a*) **From the Mouth.**—With a blunt knife, or with the finger nail, scrape off a thin shred from the mucous membrane of the cheek; mix it with a drop of normal saline solution on a slide; place on it a cover glass, and examine with a power of about 200 diameters.

The cells consist of large, flat, roundish, or irregularly polyhedral bodies, of various sizes. The substance is more or less transparent, containing granular matter. The nuclei are small, oval, frequently granular, and sometimes missing.

Preparation.—To obtain epithelial cells of various kinds, it is advisable to subject the tissues from which they are to be prepared to the following process:—Immerse in 2% solution of pot. bichromas for twenty-four hours after removal from the body, and then wash with distilled water until the washings are no longer yellow. Afterwards transfer to a mixture of equal parts of aqueous hæmatoxylin and glycerin for a day, and keep in glycerin.

(*b*) **From the Œsophagus.**—Tease a scraping from the œsophagus of a cat or dog, prepared after the above method, in a small drop of glycerin. The nuclei of the cells are seen to be stained with the dye.

COLUMNAR EPITHELIUM.

From the Intestine.—Take a small scraping from the mucous membrane of the intestine of some animal (cat, rabbit, or dog), prepared as above, and tease up with needles in a drop of glycerin on a glass slide.

The cells are cylindrical or conical in form, with a fairly well-defined outline: protoplasm finely granular; nucleus clear, oval, well-defined.

When an aggregation of cells is seen from above, as on the surface of a villus, it has the appearance of a regular mosaic.

TRANSITIONAL.

This type of epithelium is best seen in the bladder, from which it may be prepared in manner similar to the above. The shape of the cells should be noted: some are tailed, others concave on one side, spindle-shaped, or caudate. The nuclei are very large.

CILIATED EPITHELIUM.

Scrape lightly the mucous surface of a prepared trachea, tease out in glycerin, and examine in a similar manner.

The free border of each cell is provided with cilia, whilst the deeper portion is prolonged into a fine process or tail.

Study of Ciliary Motion.—With a sharp pair of scissors cut off a small fragment of one of the branchiæ of a living oyster or mussel. The epithelium scraped from the roof of a frog's mouth, or epithelium obtained from the mucus of the nose, may be employed. The epithelium lining the alimentary canal of the earthworm is also well adapted for the demonstration of ciliary motion. The examination is to be made in normal saline solution, the preparation being slightly teased previous to covering. The highest available power should be used.

Ciliary movement at first very rapid; soon becomes slower, and finally ceases.

Effects of Reagents.—Dilute alkalies slow, and then stop the movements. If the cilia are working slowly, or have stopped in a preparation which has just been put up, the careful addition of a very dilute solution of caustic potash or dilute acetic acid, or the passage over it of carbonic acid, or an electric shock, will generally renew or accelerate the movements for a short time,—the ultimate effect, however, being to destroy the cilia.

Carbonic acid first accelerates, then slows, and finally stops the ciliary action, the movements recommencing if air is allowed to take the place of the carbonic acid.

Chloroform retards and finally stops ciliary action; the movements recommencing on the admission of air, if the vapour has not been applied for too long a period.

Warmth accelerates the action of cilia which were previously moving slowly, the movements ceasing at a temperature which is sufficient to destroy the vitality of the cells.

GLANDULAR EPITHELIUM.

It is advisable to postpone the study of this form of epithelium until sections of glands, such as the liver or kidney, have been cut and prepared. The cells may, however, be seen by taking a scraping from the freshly cut surface of a kidney or liver, and preparing it in salt solution.

The cells vary in shape and size, are pale, and fairly well defined.

PIGMENT

Can be studied in scrapings from the choroid, iris, etc., teased and mounted in glycerin. The cells are either irregular and wide-branching with clear nuclei, or flattened and polygonal.

ENDOTHELIUM.

ENDOTHELIUM OF SEROUS MEMBRANES.

Preparation.—From an animal (best a cat) which has been recently killed by bleeding, take a portion of the omentum, pericardium, mesentery, or other serous membrane, and treat with silver nitrate in the way described (p. 18), and mount pieces in glycerin as there directed. The endothelial cells will by this means be mapped out. The nuclei may be shown by double staining in hæmatoxylin.

General Characters of Cells.—On examination, the surface of the membrane is found to consist of a single layer of flattened polyhedral cells, variously modified, and forming a mosaic. Nuclei generally single, and only to be detected in deeply stained specimens, appear as bright and almost colourless oval bodies within the cells. When seen in profile, the nuclei occasion a projection from the surface.

THE CONNECTIVE TISSUES.

VARIETIES.

1. White fibrous tissues.
2. Elastic tissue.
3. Adenoid tissue.
4. Gelatinous or embryonal tissue.
5. Adipose tissue.
6. Cartilage.
7. Bone.

I. WHITE FIBROUS TISSUES.

Preparation.—The tail of a newly killed young rat or mouse is cut off close to the base. The skin is removed, and a small piece of the extremity is pinched off between the nails, and is drawn away from the rest of the tail. In separating this piece, a number of fine threads, the tendons, will be noticed. One of these of moderate size is selected and teased out in saline solution.

Transverse sections of tails which have been treated with gold chloride should also be made, prepared and mounted in Canada balsam. These tails, after staining, must be left for one or two days in a weak solution of hydrochloric acid, in order that the bone may be soft enough to cut.

Structure.—The tissue is then seen to consist of parallel *bundles of fibres*, which vary in thickness, and are held together by a homogeneous and albuminous cement substance. The individual fibres forming the bundles are straight or wavy, and are extremely delicate. Acetic acid added to tendons causes *the fibres to swell up* and to disappear, owing to the presence in the tissue of a substance which is readily convertible into glutin or gelatin. The bundles of fibrils are surrounded by a more or less complete sheath of elastic tissue, which is not acted upon by dilute acids; hence the constricted appearance seen in tendons to which acetic acid has been added.

To Demonstrate the Presence of THE TENDON CORPUSCLES.

Preparation.—The most delicate of the tendons obtained from the tail of a rat is stretched, whilst it is still perfectly fresh, upon a glass slide. The extremities of the tendon are allowed to dry, and by this means it is maintained in an extended condition. A few drops of picrocarmin are placed upon the centre of the tendon, and are washed away with distilled water after the expiration of half an hour. A drop of glycerin acidified with acetic or formic acid is then added, a hair is placed by the side of the tendon to obviate pressure, and a cover glass is put on, the preparation being sealed up in the usual way. Good results can also be obtained by mounting the isolated tendons in a 1% solution of acetic acid to which ½ its volume of logwood alum solution has been added. The preparation must be examined as soon as possible. Also by treatment with a $\frac{1}{10}$%

solution of osmic acid for an hour, washing in distilled water three hours, and subsequent staining with picrocarmin.

An easier method is to place the fresh tendons in a mixture of equal parts of aqueous hæmatoxylin and glycerin for two days, tease in glycerin, and press the cover glass slightly after mounting.

Structure.—On examination, the tendons thus treated are found to consist of parallel bundles of fibres, whose substance is almost colourless, arranged in groups. Between each two groups is a lymph channel, in which lie nearly parallel layers of delicate stained cells—*the connective-tissue* or *tendon cells*—forming for each channel a single continuous row of rectangular plates. Each plate is provided with a more deeply staining nucleus. The cells are separated from each other by a cementing substance, and they possess fine processes. Each cell presents a straight ridge—*the elastic stripe.* This ridge is formed by the union of two or three concave portions of which the cell is composed, to enable it to adapt itself to the curved surfaces of the tendon bundles. *The lymphatics* may be demonstrated by staining the tail of a very young rat in chloride of gold, and then making fine transverse sections; dark masses will then be seen in the tendon corresponding to the *lymphatic channels*, filled with an albuminous fluid plasma. Radiating from these masses are fine septa—*the cement substance*—binding together the contiguous bundles.

AREOLAR TISSUE.

Preparation.—This form of tissue may be called a variety of fibrous tissue, and is best seen in specimens of intestine and skin. A small artificial bulla is formed in a rat, which is still warm by the injection into the subcutaneous tissue of a 0·2% solution of nitrate of silver or osmic acid, which is allowed to remain for ten to thirty minutes. The bulla is then opened with a pair of fine curved scissors, and the delicate subcutaneous tissue is rapidly removed and spread out on a glass slide. It is immediately covered with a thin glass, and the preparation is stained for twenty-four hours with picrocarmin. Glycerin is passed through until all the superfluous staining material is removed, after which the preparation is sealed up.

Structure.—The tissue is composed of delicate bundles of

ordinary white fibrous tissue, some of the fibres are fibrillated, and all interlace with each other; the meshwork thus formed contains a few very fine fibrils of *elastic tissue*. The interspaces are filled with lymph-containing *lymph corpuscles*. Large *plate-like cells*, which appear to lie upon the surface of the bundles of fibres, are also seen. When viewed sideways, these cells have a branched appearance, and form the *plasmatic cells*. *Fat cells* are also present.

II. ELASTIC TISSUE.

Preparation.—Tease out a small piece of the ligamentum nuchæ of an ox in glycerin, and examine.

Structure.—Elastic fibres are *thick* and well defined, and form bundles; or they are fine, shining, and unconnected in bundles. They *branch* dichotomously, and *anastomose* with each other to form a real network; when torn, they *curl up* at the ends. They do not swell up when treated with acids, and they yield elastin.

III. ADENOID TISSUE,

Which forms the basis of the spleen, the lymphatic glands, the tonsils, thymus, Peyer's glands, etc., is a fine reticular tissue, which is composed of the branchings of corpuscles uniting to form a meshwork, the corpuscles either retaining or not retaining their nuclei, or of a finely felted meshwork of endothelial plates.

IV. GELATINOUS, EMBRYONAL, MUCOUS, OR WHARTON'S TISSUE.

Preparation.—Present in the umbilical cord, and in the fœtal skin. The connective tissue is obtained from a bulla formed by the injection of a dilute solution of gold chloride, in a stronger solution of which it is subsequently stained.

Structure.—A transparent jelly-like substance in the youngest condition, containing a hyaline mucous substance within a reticular framework. At a later period *bundles of fibrous connective tissue* are apparent, as well as *branched cells*, *blood-vessels*, and *fat cells* in an early stage of development. The tissue yields mucin on boiling. *The vitreous humour* appears to be a variety of this tissue, in which the branched cells have lost their processes.

V. ADIPOSE TISSUE.

Preparation.—May be seen best in sections of scalp, etc. Tease out a small piece of fat in glycerin. Leave a small piece of fat, which has been partially teased, in ether for twenty-four hours, and the fatty portion will be dissolved out. Examine also the preparation of areolar tissue formed by the injection of nitrate of silver. Sections of fat stained with osmic acid should also be examined.

Structure.—Adipose tissue consists of a matrix or network of areolar tissue containing fat cells. *Fat cells* are clear, well-defined, rounded vesicles of varying size, filled with an oily fluid, which often gives rise after death to *crystalline needles*, probably of margarin, radiating from the centre of the cell. In successful preparations a fine zone of *protoplasm*, with a *nucleus* at one pole, can be seen surrounding the cell more or less completely. The fat cells may either form compact masses, with only a small amount of connective tissue, or they may be more or less isolated. The tissue possesses a capillary network of *blood-vessels*. Between the fat cells, flattened nucleated *connective-tissue cells* may be demonstrated.

VI. CARTILAGE.

CARTILAGE consists of two parts:

1. Cells.
2. Matrix or intercellular material.

According to the nature of the matrix, cartilage is classed as—

1. Hyaline cartilage.
2. Fibro-cartilage.
3. Elastic cartilage.

I. HYALINE CARTILAGE.

Preparation.—Hyaline cartilage is found in various localities, from which the names, costal, tracheal, articular (from the articular surface of bone), ossifying or intermediary, and embryonal, are derived. Portions of each of these cartilages should therefore be examined. The cartilages may be prepared in a solution of chromic acid 1 in 600, in a saturated solution of

picric acid, or by the gold method. In every case the sections, which must be very thin, should be stained with carmine or hæmatoxylin.

It may be as well also to stain some of the nasal cartilage in osmic acid.

Structure.—All cartilage, with the exception of the free extremity of articular cartilage, possesses a delicate vascular connective-tissue sheath—*the perichondrium. The cartilage cells* are spherical or oval protoplasmic bodies, generally containing a single nucleus. The cell protoplasm forms a fibrillar meshwork which is contracted in embryonal and articular cartilages. Each cell is placed in a lacuna, enclosed by a firm, structureless, but transparent *matrix*, yielding chondrin. In growing cartilage, a special layer—*the limiting membrane*—can be distinguished between the lacuna and the ground substance. In some cases a single lacuna may contain more than one cell, due to reproduction of the cartilage cell by fission; and the various stages in the division of cells may often be well seen. Near the perichondrium the cartilage cells become flattened and smaller; near the articular surface they are branched; in ossifying cartilage they are arranged in parallel rows. The matrix possesses an anastomosing system of lacunæ and canals in connection with the *lymphatic* system.

II. FIBRO-CARTILAGE.

Preparation.—As for hyaline cartilage.

White fibro-cartilage occurs in the intervertebral substance and in sesamoid bones.

Structure.—It consists of groups of slightly flattened elastic *cells*, each with a round nucleus, and enclosed in a distinct capsule. The matrix is composed of bundles of fibrous tissue, which sometimes form lamellæ with occasionally a concentric arrangement.

III. ELASTIC CARTILAGE.

Preparation—As for hyaline cartilage.

This form of cartilage occurs in the lobe of the ear, in the epiglottis, in the cornicula laryngis, in the cartilages of Wrisberg and Santorini, and in the Eustachian tube.

Structure.—Elastic cartilage in the adult is hyaline car-

tilage permeated by elastic fibrils. *The fibrils* are arranged so as to form the trabeculæ of a reticular framework; they branch and anastomose very frequently. The meshes contain fusiform groups of large nucleated *cells*, surrounded by a larger or smaller amount of hyaline cartilage substance.

IV. OSSIFYING OR CALCIFYING CARTILAGE.

Preparation.—The fœtal femur or other long bone is prepared by decalcifying with the chromic acid and hydrochloric acid mixture, and cutting sections both transverse of the shaft and longitudinal of the extremities. The sections may be doubly stained in picrocarmin (or eosin) and hæmatoxylin. This variety of cartilage is found at the junctions of cartilage with spongy bone in the epiphyses and ends of the shafts of long bones, and represents the method by which bone increases in length. It will be seen that the stages of the formation of bone in this way correspond almost exactly with the so-called development of bone in cartilage. In a section through the end of a growing long bone the following layers can be seen (Klein) at the junctions of the cartilage and bone :—

(1) Ordinary hyaline cartilage covered with perichondrium in the diaphysis of a long bone at the junction of the cartilage with the spongy bone, a characteristic arrangement of *the cartilage cells* in longitudinal columns. The cells are also seen to be conical in shape, pressed together and flattened transversely.

(2) A *transparent layer*, in which the lacunæ of the cartilage cells are seen to be enlarged, the matrix diminished, the cells enlarged and transparent, and their nuclei swollen.

(3) The lacunæ are becoming confluent, and the matrix calcified.

(4) The enlarged lacunæ are seen to be filled with marrow, and the trabeculæ of calcified cartilage are covered with layers of marrow cells, amongst which are enlarged multi-nucleated mother-cells (giant cells).

(5) The marrow cells (osteoblasts) are seen to have deposited layers of low *ossific material* upon the calcified trabeculæ, and at the same time the calcified centres have become less free from absorption.

(6) The calcified centres of the trabeculæ have disappeared, leaving ossific trabeculæ which form the spongy bone.

VII. BONE.

Preparation.—The fresh bones of any small animal, well cleared of the surrounding tissues, are to be placed for two or three weeks in a large quantity of $\frac{1}{2}$% solution of chromic acid, containing five drops of hydrochloric or nitric acid to each ounce of the solution. When the whole of the earthy matter is dissolved out, sections are to be cut with a razor in various directions, and examined in glycerin.

As the preparation of specimens of hard bone requires much time, the student is advised to buy both transverse and longitudinal sections of that material.

General Characters.—A. *Compact bone.*—In transverse sections of the compact tissue of long bones are seen Haversian systems, more or less perfect, and Haversian interspaces. Each system consists of the central *Haversian canal*, which is generally round or oval, with an average diameter of $\frac{1}{100}$ in., and is lined with a delicate membrane continuous with the periosteum, surrounded by concentric lamellæ of bone, in which are the lacunæ and canaliculi. *Lacunæ* $\frac{1}{1000}$ in. in length, generally well marked, contain shrunken bone corpuscles. *Canaliculi* usually indistinct, but when seen plainly, forming a complete system of communication between the lacunæ of the same and neighbouring Haversian systems and interspaces. They contain in the fresh condition prolongations from the bone corpuscles. Each Haversian system is more or less isolated from its neighbour by a layer of bone which contains but few canaliculi. The *Haversian interspaces* are the portions of bone filling up the interval between one or more of the circular Haversian systems. They do not contain any central canal; their general characters are otherwise similar to the systems.

In longitudinal sections the Haversian canals which run longitudinally are seen to anastomose freely by transverse or oblique channels. The lacunæ and canaliculi present much the same characters as in transverse sections. The Haversian canals which run near the circumference of the bone may open on the outer surface so as to admit blood-vessels from the periosteum, whilst those opening into the medullary canal receive blood-vessels, and in the case of the larger ones, medulla from the interior of the bone.

In preparations of calcified bone it may be seen that the lamellæ are bolted together by *the perforating fibres of Sharpey*. Bone situated immediately beneath an articular cartilage differs in not possessing Haversian canals, in the lacunæ being larger than in ordinary bone, and in being destitute of canaliculi. *The periosteum* covering the free surface of bones consists of an external layer of dense fibrous tissue supplied by capillary blood-vessels, and an internal osteo-genetic layer containing a plexus of delicate connective-tissue fibrils; in the meshwork formed by these fibrils are capillary blood-vessels and a number of nucleated cells. *The medulla* is of the yellow kind, and is chiefly composed of fat cells, with intervening membranes of flattened connective-tissue cells; it also contains numerous cells possessing one or two nuclei.

B. *Spongy or cancellous bones.*—In spongy bones—*e.g.*, a vertebral, carpal, or tarsal bone—the tissue consists of bone trabeculæ, forming a more or less open framework, in which is embedded the medullary substance. Bone trabeculæ contain lacunæ with bone corpuscles and ill-developed canaliculi. The medullary substance is of the red kind; it is rich in blood-vessels, and in cells having the characters of lymph corpuscles, fat cells, etc.

THE DEVELOPMENT OF BONE.

It is usual to describe two ways in which bone is developed, viz., (1) in cartilage, (2) in membrane, but in reality the shaft of all permanent bones is developed in membrane, as will be seen in the following description. The so-called development in cartilage is really a preliminary stage, and all bone which is so formed, except at the growing ends, does not remain a part of the permanent bone, but is reabsorbed nearly as soon as it is made.

In the account of the formation of bone in cartilage, the stages under which the process is described are more or less arbitrary, being inserted for the convenience of description.

1. **Development of Bone in Cartilage.**—Stage i. *Hyaline cartilage* covered by perichondrium. *The perichondrium* consists of an outer layer of embryonal connective tissue, and an inner osteogenetic layer containing spherical cells—the future osteoblasts and blood-vessels. Stage ii. *The inner layer of peri-*

chondrium penetrates the cartilage, forming for itself channels by absorption, and carrying with it blood-vessels and cells. The growth of the perichondrium inward starts at the centres of ossification. Stage iii. *The primary marrow cavities* are formed by the appearance of lacunæ near the cartilage channels, which then become confluent, whilst the trabeculæ separating neighbouring lacunæ become calcified. The primary marrow filling the marrow cavities is the periosteal ingrowth containing the vessels and cells. Stage iv. *The calcified trabeculæ become ensheathed with osseous material*, and are then absorbed. A network of osseous trabeculæ instead of a network of calcified cartilage is thus formed, whilst the whole tissue resembles spongy bone (endochondral bone). The surfaces of the osseous trabeculæ are covered with osteoblasts, whilst the cavities separated by the trabeculæ are filled with marrow rich in vessels and cells. So far we have almost the same stages as described above, under the head of ossifying cartilage. Stage v. The endochondral spongy bone is absorbed, from the centre outwards; the large *medullary cavity* is thus formed. *Bone from the periosteum* is also simultaneously developed round the endochondral bone. *The osteoblasts* multiply and become converted into the osseous matrix and into bone corpuscles. The meshes of the spongy periosteal bone are *the Haversian spaces;* they contain marrow from which a series of concentric lamellæ are formed. The spaces are thus gradually reduced to *Haversian canals.* Stage vi. *All the endochondral bone is absorbed*, and the ossified trabeculæ are represented by the interstitial substance separating the concentric Haversian lamellæ.

As the bone increases in thickness, though continued by the formation of new bone on the outside by the periosteum, the primary periosteal bone is pushed towards the centre and is absorbed, first of all becoming more spongy in its nature. The bone formed later from the periosteum being much denser and more compact in structure than that first formed.

2. **Development of Bone in Membrane.**—The membrane corresponds to the future periosteum; it consists of two parts, as above. Stage i. The cells of the osteo-genetic layer—*the osteoblasts*—increase and form the osseous matrix by excreting ossein around them and the bone corpuscles, thus forming ossified trabeculæ which start from the centres of ossification.

Stage ii. Portions of the trabeculæ are absorbed, whilst, as in endochondral bone, concentric lamellæ are formed by the marrow in the Haversian canals.

The formation of intramembranous bone is identical with the formation of periosteal bone. The absorption of osseous substance is in nearly every case associated with the presence of multinucleated giant cells—*the osteoclasts.*

Transverse Section of the Tail of a Rat.—The central portion is the bone, fully ossified or not, according to the age of the animal from which the preparation was made. The medulla, Haversian canals, bone corpuscles, and, if the animal was young, the ossifying cartilage, should be drawn under a low power. Surrounding the bone is the periosteum. A layer of fat or muscle, with nerves and blood-vessels, lies external to it, between the periosteum and the tendons of the tail. The tendons consist of a number of bundles cut transversely, in the substance of each of which are seen numerous tendon corpuscles. Between the tendon bundles is fat. Still more externally, and constituting the outer margin of the preparation, is the connective tissue which formed a part of the corium before the skin was stripped off.

MUSCULAR TISSUE.

VARIETIES.

1. Striated or striped. 2. Plain or unstriped.

Preparation.—For the examination of fresh muscle, tear away a small fragment of the tissue with a pair of forceps from a recently killed cockroach or water-beetle (*Dytiscus marginalis*), and at once tease it up thoroughly in a drop of salt solution or water on a glass slide. Examine as soon as possible.

Muscle is also prepared by taking small pieces of fresh tissue from the voluntary muscles and the heart, as well as from any of the viscera containing unstriped fibres, and soaking them for a few weeks in a 2% solution of chromic acid, or in a $\frac{1}{10}$% osmic acid for one hour, then washing in distilled water for two hours. A fragment of the tissue is removed, and, having been

well teased with needles, is examined in water, glycerin, or other medium.

Unstriped tissue is prepared by distending a piece of rabbit's intestine with saline solution, leaving it in a 1% solution of anilin black for twenty-four hours, and stripping off the outer coat with forceps. Small pieces of this coat are then to be mounted in glycerin.

In addition to the above preparation, it is as well to examine sections from the tongue which have been stained in hæmatoxylin, passed through the ordinary reagents, and mounted in Canada balsam, and others from the muscular coat of the stomach or intestine, treated in a similar manner, in order to see stained striped and unstriped fibres respectively.

GENERAL CHARACTERS.

1. Striped Muscular Tissue. *From Voluntary Muscle.* —Consists of long *fibres*, which are cylindrical, but appear in transverse section as rounded polygons. Each fibre is made up of a number of exceedingly fine and delicate filaments, *the fibrillæ*, enclosed within the sarcolemma. The fibres are aggregated into *bundles;* several bundles forming *fasciculi*, and these the anatomical *muscle*. *Perimysium* or fibrous connective tissue surrounds the bundles; from it pass off small processes of connective tissue, with cell plates and plasma cells, between the muscle fibres—*the endomysium*. Each fibre consists of broad dim bands of highly refractive substance representing the contractile portion of the muscle fibre—*the contractile discs*—alternating with narrow bright bands of a less refractive substance —the *interstitial discs*. After hardening, each contractile disc becomes longitudinally striated, the thin oblong rods thus formed being the *sarcous elements* of Bowman. The sarcous elements are not the optical units, since each consists of minute doubly refracting elements—*the disdiaclasts* of Brücke. When seen in transverse section, a muscular fibre appears to be subdivided by clear lines into polygonal areas—*Cohnheim's fields*, each corresponding to several sarcous element prisms. The clear lines are due to a transparent interstitial fluid substance pressed out of the sarcous elements when they coagulate. *The sarcolemma* is a transparent structureless elastic sheath of great

resistance, which surrounds each fibre. From the sarcolemma, transverse membranous septa—*the membranes of Krause*—extend inwards across the muscle at regular intervals. By these septa the muscle fibre is divided into equal-sized *muscle compartments*, each containing one contractile disc. The membranes of Krause are so placed that each passes across the middle of an interstitial disc, which is thus divided into two *lateral dics.*

A thin transverse median disc—*the disc of Hensen*—is occasionally seen to divide the contractile disc. In some fibres, chiefly those from insects, each lateral disc contains a row of bright granules forming *the granular layer* of Flögel. The fibres contain nuclei, which are roundish, ovoid, or spindle-shaped in different animals. These nuclei are situated close to the sarcolemma, their long axes being parallel to the fibres which contain them. Each nucleus is composed of a uniform network of fibrils, and is embedded in a thin more or less branched film of protoplasm. The nucleus and protoplasm together form the muscle cell or *muscle corpuscle* of Max Schultze.

The arteries and veins are in the perimysium; the capillaries in the endomysium, between the fibrillæ. The capillary plexus is elongated in shape. The individual capillaries run parallel with the muscle fibres, and anastomose with each other by short branches.

Muscular Tissue of the Heart.—Fibres very small and finely striated. Striæ generally indistinct, often showing only as coarse granules. There is no sarcolemma. Many of the fibres anastomose and branch. The nuclei are in the centre of the fibres.

2. Non-striped Muscular Tissue.—Made up of bundles of cells, bound together by an albuminous cementing substance—the *endomysium*—in which lie connective-tissue cells and a few fibres. The *perimysium* continuous with the endomysium is the fibrous connective tissue surrounding and separating the bundles of muscle cells. Fibres, fusiform, band-like, or spindle-shaped, containing elongated or staff-shaped nuclei, placed midway in the fibres. The ends may be split into two or more parts, as may frequently be seen in the cells obtained from arteries and veins. Each *muscle cell* consists of a fine sheath, probably elastic; of a central bundle of fibrils representing the contrac-

tile substance; and of an oblong nucleus, which includes within a membrane a fine network anastomosing at the poles of the nucleus with the contractile fibrils. Ends of fibres usually single, sometimes divided. There is no sarcolemma.

NERVE TISSUE.

VARIETIES.

1. Nerve fibres { Medullated. / Non-medullated.
2. Nerve cells.

Preparation.—Cut longitudinal sections of a portion of a sciatic nerve, which has been hardened for about ten days in a 2% solution of bichromate of potassium or a mixture of spirit and ¼% chromic acid in equal parts. Stain these deeply in hæmatoxylin solution, and tease them out, then mount in glycerin. Cut also transverse sections, stain, prepare, and mount in Canada balsam.

Take a fresh nerve, *e.g.*, the sciatic of a rabbit, guinea-pig, rat, or frog, cut it into short lengths, and place portions quickly in (1) osmic acid, (2) gold chloride, (3) silver nitrate, (4) hæmatoxylin or carmine. Tease the pieces slightly whilst in the reagents. Treat the gold, silver, and osmic acid pieces according to the method described (pp. 19 and 20), and tease a small strand of each; mount in glycerin. The osmic acid demonstrates chiefly the medullary sheath; the silver stain, the endothelium covering the nerve and the nodes of Ranvier; and the other reagents, principally the axis cylinders.

The auditory, sympathetic, and optic nerves may also be examined by teasing, and in transverse section after hardening and staining.

1. Nerve Fibres.—(*a*) *Medullated Nerve Fibre. From Spinal Nerves.* The nerve trunks are composed of a variable number of bundles of nerve fibres (*funiculi*), which have a special sheath (*perineurium* or *neurilemma*), and are enclosed in a firm fibrous sheath (*epineurium*), which also sends in processes of connective

tissue, connecting the bundles together. With the funiculi, between the fibres, is a delicate supporting tissue (the *endoneurium*).

Each nerve fibre is made up of the following parts :—

(1) *Primitive nerve sheath*, or *nucleated sheath of Schwann.* (2) *Medullary sheath*, or *white substance of Schwann.* (3) *Axis cylinder, primitive band, axis band*, or *axial fibre.*

Schwann's sheath, which is the external layer of the fibre, appears to be a simple, transparent, colourless, homogeneous structure, with a varied number of oval nuclei attached. It probably consists, however, of nucleated endothelial cells joined end to end, and forming a complete membrane.

The *medullary sheath*, the middle layer, which forms the greater part of the nerve, surrounds the axis cylinder, and has a double contour ; and at regular intervals are constrictions in the medullary sheath (*Ranvier's constrictions*), caused by the thinning or interruption of the medullary substance. In it may be demonstrated, by the action of osmic acid, the *sections of Lantermann*, of which the medullary sheath is said to be made up.

The *axis cylinder* is situated in the middle, and appears as a faintly marked band, with an exceedingly fine and even outline, and is made up in the prepared specimen of a number of most delicate fibrils (*primitive fibrils*).

Between the axis cylinder and the medullary sheath there is said to be a small space containing in the recent state albuminous fluid. At the constrictions of Ranvier, in nerves stained with silver nitrate, a black cross is seen, due to the action of the silver on the albuminous cement substance between the constricting folds, and to its penetrating and staining the cement substance surrounding the axis cylinder for a variable distance.

From Cerebro-spinal Centre, the Optic and Auditory Nerves.—The general appearances are the same, excepting that there is no sheath of Schwann. These nerves, however, are not made up of distinct funiculi.

(*b*) *Non-medullated Nerve Fibre. From Sympathetic* and Olfactory Nerves.*—Consists of simple filaments forming an axis cylinder, and surrounded by a nucleated sheath of Schwann.

2. Nerve cells.—(Are examined with the brain and spinal cord.)

* See also p. 68.

containing a few small cells with fine processes embedded in a considerable quantity of neuroglia. This layer composes about $\frac{1}{10}$ of the whole thickness of the grey substance. (2) *The second layer* of small, densely aggregated, pyramidal cells, provided with branching processes. This layer is of nearly the same extent as the previous one. (3) *The third layer* is of greater width, and is somewhat paler than the first and second layers; it is composed of large and small pyramidal cells, arranged with their apices turned towards the surface of the convolution. The larger cells average $\frac{1}{1800}$ inch across their base. The cells are arranged in groups, and are separated from each other by bundles of radiating nerve fibres, each bundle being about $\frac{1}{1800}$ inch in diameter. The pyramidal cells send downwards three processes, of which the middle one forms an axis cylinder. Both cells and processes are striated longitudinally, and generally contain a little yellowish pigment. (4) *The fourth layer* is somewhat narrower than the preceding; it consists of small, irregularly placed, granule-like corpuscles, with delicate processes. The cells are less distinctly separated into groups. (5) *The lowest layer* is of considerable width; it contains, in addition to cells resembling those of the fourth layer, fusiform cells arranged vertically at the summit of a gyrus, but parallel to the surface of a sulcus. This layer gradually blends with (B) *The white substance*, composed essentially of white nerve fibres, which are smaller than those of the spinal cord, with an average diameter of $\frac{1}{10000}$ inch. In the neighbourhood of the cortex, a few non-medullated fibres can be seen. (C) *The neuroglia*, formed of a homogeneous matrix, in which lie numerous elastic fibrils, connected into a network. With this network the branched nucleated cells of Deiter are connected.

CEREBELLUM.

Preparation.—The cerebellum is hardened in the usual bichromate mixture. To stain specimens of cerebellum, the following method is recommended. Thin sections should be left in a ·01% solution of eosin for twelve hours, and then, after washing in distilled water, slightly acidulated, should be placed in a weak solution of anilin green for fifteen or twenty minutes, and afterwards should be passed rapidly through the ordinary reagents, and mounted as for cerebrum.

Structure.—Like the brain, it is divisible into (A) Cortical grey; and (B) Internal white substance. *The Cortex* is divisilbe into (1) *The molecular layer*, the most external, consisting of a nerve network containing small pear-shaped multipolar ganglion cells. The fibres of the network in the more superficial portions are nearly vertical to the surface; they are derived partly from the neuroglia, partly from the processes of the cells of Purkinje. (2) A single layer of large spindle-shaped ganglion cells $\frac{1}{500}$ to $\frac{1}{1000}$ inch in diameter, *Purkinje's cells.* Each cell possesses one branched process which extends into the molecular layer, where it branches dichotomously, some of the finest ramifications looping backwards to terminate in the granular layer, and an unbranched axis cylinder process passing downwards. The cells lie in a pericellular space, and each consists of a minute network of fibrils extending into the branched processes. The nucleus is spherical and oval. (3) *The granular layer*, containing a network of minute fibrils and dense groups of granule-like corpuscles. These corpuscles average $\frac{1}{4000}$ to $\frac{1}{5000}$ inch in diameter. (B) *The medullary centre*, or *internal white substance*, consists of nerve fibres arranged in parallel or interlacing bundles.

The neuroglia of the white matter contains rows of small cells, each with a spherical nucleus, between bundles of nerve fibres.

The blood-vessels of the grey matter pass from the pia mater in a vertical or oblique direction, and anastomose into a uniform network. The blood-vessels of the white matter form a network with longitudinal meshes. The vessels lie in lymph channels, *the perivascular lymphatics* of His.

SYMPATHETIC SYSTEM.

Preparation.—Harden (1) portions of the Gasserian ganglion from the sheep in chromic acid and spirit. (2) Pieces of the sympathetic nerve from the neck of the ox, in the manner recommended for medullated nerve fibres. (3) Pacinian corpuscles by snipping out pieces of the mesentery or mesorectum of the cat, in which they may be seen as small bodies embedded in the fat: isolate them with needles, treat with osmic acid, stain in picrocarmin, and mount in glycerin. (4) Meissner's and Auerbach's plexuses, situated the one in the submucous

coat, and the other between the muscular coats, of the intestine, are best prepared from the rabbit or guinea-pig. A piece of intestine, three inches in length, is distended with the juice of a fresh lemon, the ends being ligatured; it is allowed to remain in the lemon juice for about five minutes. The ligatures are then removed, and it is washed thoroughly in water, and filled with a 2% solution of gold chloride; it is again ligatured, and is then suspended for half an hour in a 1% solution of gold chloride, washed thoroughly, and transferred to a 24% solution of formic acid, to reduce the gold, the preparation being kept in the dark. After reduction of the gold, the intestine is of a rich reddish-brown colour: it should be again washed. Peel off strips of the outer muscular coat with forceps, and mount in glycerin. Auerbach's plexus will be seen (Stirling). The intestine may be stained with hæmatoxylin, if thought advisable. The plexus of Meissner is demonstrated by treating the intestine as before, and afterwards inverting it and removing the mucous coat, when portions of the submucous coat may be picked off from the muscular coats, and mounted in glycerin.

General Characters.

The ganglion cells are of various shapes and sizes, generally smaller than the cells of the cerebro-spinal ganglia; they possess a capsule, and consist of a network of fibres. There is usually one large, excentric, oval nucleus, which may, however, be double. The cells possess one or more processes, which are continuations from the cell substance, and are invested by a prolongation from the hyaline sheath. According to the number of processes, the cells are unipolar, bipolar, or multipolar. In the frog the bipolar cells are peculiar, since one of the processes appears as a "spiral fibre" twisted round the other process, or "straight fibre."

The nerve trunks contain medullated fibres similar to those already described, and non-medullated, or fibres of Remak. Remak's fibres are pale, finely fibrillated axis cylinders, invested with a hyaline sheath of Schwann provided with nerve corpuscles.

The Pacinian bodies are oblong corpuscles connected with a medullated nerve fibre which represents its stalk. The corpuscle consists of a number of concentric capsules. Each

capsule is composed of a hyaline basement membrane, which is probably elastic, and in which are embedded fine connective-tissue fibres. On the inner surface of the basement membrane is a layer of flattened nucleated endothelial membrane, which is visible after treatment with nitrate of silver. In the centre of the corpuscle is a clear mass in which lies the axis cylinder of the perforating nerve fibre. The axis cylinder generally breaks up into two or three branches, or it may terminate in a bud or in a pointed or fringed extremity. The corpuscles contain, between the capsules, capillary blood-vessels and a few plasma cells.

The nerve plexuses consist of a meshwork of flat nerves, each of which is ensheathed in a delicate endothelial membrane: at the nodal points are groups of ganglion cells, which vary in size and shape; the larger ones possess a capsule and processes. The plexus of Meissner consists of a larger and less regular meshwork than that of Auerbach. The two plexuses are connected by branches which pass through the circular layer of muscle. Each plexus gives off branches which supply the surrounding tissues.

BLOOD-VESSELS.

Varieties.—Of three kinds. (A) Arteries, (B) Veins, and (C) Capillaries.

(A) ARTERIES.

Preparation.—*Longitudinal* and *transverse* sections of a medium-sized artery (or vein), which has been hardened in a 1% solution of potassium bichromate, should be stained in logwood, prepared, and mounted in Canada balsam.

Structure.—Arteries (except those of minute size) have three coats:—

1. Internal coat, consisting of (*a*) *An epithelial layer*, forming the lining of the vessel, of thin elliptical or irregularly polygonal cells, often lanceolate, with nuclei and nucleoli; (*b*) *A sub-epithelial layer* of delicate connective tissue, with branched corpuscles; (*c*) *Elastic layers* of longitudinal elastic networks

and "fenestrated" membrane. 2. Middle coat chiefly consists of circular *unstriped muscle fibres*, mixed with *elastic fibres*, and a sparse amount of connective tissue. 3. External coat (*tunica adventitia*) chiefly consists of fine and closely felted bundles of *connective tissue*, together with longitudinal elastic tissue between them.

In the largest arteries, the middle coat consists of alternate layers of elastic tissue and unstriped muscle. In the smallest arteries (arterioles) the coats are reduced to a muscular, principally of circular fibres, and a lining endothelium.

(B) VEINS.

Preparation.—As of the arteries.

Structure.—As of the arteries, with these differences: The elastic tissue of the internal coat seldom occurs in the form of fenestrated membranes. The middle coat is thinner, contains less muscular tissue and more white connective tissue. The external coat of some veins has a considerable amount of unstriped muscular fibre.

(C) CAPILLARIES.

Preparation.—These are to be obtained from the pia mater. The brain of some animal, such as a cat or dog, should be left for two days in a 2% solution of potassium bichromate, then the pia mater may be stripped off in pieces, stained, and mounted in the usual manner. Capillaries are well seen also in the mesentery of a cat or other animal.

Structure.—The walls of the capillaries proper are formed entirely of a simple epithelial layer of flattened lanceolate cells, joined edge to edge, and continuous with the layer which lines the arteries and veins. The larger capillaries have an outside structureless or finely fibrillated coat. In vessels rather larger (small arteries and veins) there is added, outside the epithelium, a thin layer of unstriped muscular fibre.

ALIMENTARY CANAL AND ITS APPENDAGES.

(A) TONGUE.

Preparation.—Vertical sections of the tongue of a rabbit or cat, which has been hardened in equal parts of ½% chromic acid and spirit, should be stained and treated in the usual manner.

Sections of the *papilla foliata* which is found on either side of the base of the rabbit's tongue should also be treated in a similar manner. The tongue furnishes excellent material for double or triple staining.

Structure.—Consists of three layers :—

1. *The mucous coat* is covered with stratified epithelium, and is provided with papillæ (of three kinds in the human subject—viz., circumvallate, fungiform, and filiform), and these again with small, closely set secondary papillæ, which are hidden under the epithelium. The secondary papillæ are found everywhere in the mucous membrane, and not over the larger papillæ alone ; but these latter are confined to special parts, and, as a rule, are placed in circular depressions. Surrounding some of the papillæ, notably the circumvallate, are certain ovoidal or flask-like bodies, the so-called 'taste-buds,' composed of modified epithelium. Nerves are supposed to terminate in these cells. In the mucous membrane also are small tubular glands, some secreting mucus ; but others, whose ducts open into the trenches around the taste organs, secreting a more watery fluid. Lymphoid tissue, which here and there forms distinct follicular glands, is found in large quantity in the mucous membrane at the posterior part. Crypts or recesses, too, are found, the walls of which are studded with nodules of lymphoid tissue. 2. *The submucous coat* is incomplete and scanty. 3. *The muscular* portion consists of longitudinal, transverse, and vertical bundles of striated muscle.

(B) THE TEETH.

Preparation.—(*a*) By means of sections of the hard tooth : Grind down the tooth on both sides till it is quite thin, then

mount in hard Canada balsam, so as to retain the air in the various cavities. (*b*) By means of sections of the softened tooth: Place the tooth in 10% solution of hydrochloric acid till it is quite soft, then immerse in spirit; by this means the structure of the dentinal substances may be investigated; or place the tooth (preferably broken across) in a saturated solution of picric acid until quite soft. Complete the hardening in spirit, changing the spirit so long as it becomes tinged with the picric acid. This method of preparation will preserve the pulp and odontoblasts. To demonstrate the pulp, break a freshly extracted tooth, and immerse in osmic acid $\frac{1}{2}$% for twenty-four hours. To show the development of teeth, place the lower jaw, cleaned of the muscles, of fœtal rat, dog, or kitten, in $\frac{1}{2}$% chromic acid for seven days, then remove to weak spirit for twenty-four hours, and finally to strong spirit till required. (*c*) The dentinal sheaths lining the tubules may be isolated by boiling for ten minutes in strong sulphuric acid. In each case stain in hæmatoxylin.

[*Note.*—It is best to buy prepared sections of teeth.]

Structure.—(1) A tooth consists most externally, and at the surface, of *enamel.* In the recently cut tooth, which has not been used, there exists above the enamel a covering of epithelial or horny nature (Nasmyth's membrane), which is structureless, and has a thickness of $\frac{1}{3000}$ to $\frac{1}{1000}$ in. The enamel covers the crown and neck of the tooth; it is an epithelial product, consisting of closely aggregated polyhedral cylinders (prisms or columns). The enamel fibres are crossed by a number of darker lines, arranged in concentric layers, "contour lines." In transverse section, the enamel fibres are seen to be six-sided prisms, with an average diameter of $\frac{1}{2000}$ in. (2) *The Dentine* covers the body and root of the tooth; on the surface it lies immediately below the enamel. The dentine consists of a compact bone-like substance, which contains no bone corpuscles, and is permeated by dichotomously dividing canals, the dentinal tubules, which average $\frac{1}{1500}$ in. in diameter. The dentinal tubules run perpendicularly to the surface of the pulp cavity, into which they open by their lower extremities. The tubules present a proper wall, consisting of a membranous tube, and each contains a process of protoplasm from the superficial layer of the pulp cells. Examined under a lower power, the tubules

are seen to form two or three gentle curvatures, which give rise, when a number of tubules are seen together, to a series of concentric lines (lines of Schreger). Certain interglobular spaces, due to imperfect deposition of salts, are also frequently seen in the dentine. (4) *The crusta petrosa*, or cement, invests the portions of the tooth which are not protected by enamel. It closely resembles bone in its histological appearances, except that the lacunæ and canaliculi are larger and more irregular. When the cement is very thick, it may contain vascular channels, which are comparable with Haversian canals. The perforating fibres of Sharpey are present in considerable numbers in the ivory. (5) *The pulp* occupies the central cavity of the tooth; it consists of jelly-like connective tissue, in which run nerves and blood-vessels. The outermost layer of cells, forming the pulp, are elongated in form, the bodies somewhat resembling columnar epithelium cells. This layer forms the membrana eboris; each cell is an odontoblast. The odontoblasts send off one or more processes, which run in the dentinal tubules; processes which connect the cells together laterally, and processes which unite the cells to others lying more deeply. (6) *Osteodentine*, or secondary dentine, is the hard substance deposited on the inner surface of the dentine, which is produced by the gradual corrosion of the pulp.

DEVELOPMENT OF TEETH.

Preparation.—A fœtal or new-born rat is decapitated, and its head is placed in a large excess of ¼% chromic acid for a week; it is afterwards transferred to spirit. The lower jaw may then be removed, and embedded in the ordinary way; the sections should be stained in hæmatoxylin and in carmine.

(1) The first rudiment of a tooth appears as a solid prolongation of the stratified epithelium, which grows downwards from the surface into the mucous membrane. This process of epithelium is the *primary enamel organ.* (2) The enamel organ becomes invaginated at its deep end by a mass of tissue derived from the mucous membrane, called the embryonal tooth papilla. The primary enamel organ is thus converted into the *enamel cap* covering the tooth papilla. (3) *The papilla* is vascular, and is composed of a network of nucleated cells; it forms the pulp, and by means of its odontoblasts forms the dentine. (4) *The*

odontoblasts appear on the papilla as a peripheral stratum of large cells arranged vertically. (5) *The dentine* is formed by the elongation and subsequent calcification of the distal extremities of the odontoblasts, whilst (6) *the dentinal fibres* are derived from processes of cells wedged in between the odontoblasts. (7) *The tooth sac*, or the mucous membrane which immediately surrounds the enamel cap and tooth papilla, gradually grows over the former, and separates it from its connection with the surface epithelium. (8) The *enamel cap* consists externally of (*a*) columnar cells, more internally of (*b*) polyhedral cells, followed by (*c*) flattened epithelial cells in the centre, and again of (*d*) polyhedral, with (*e*) columnar cells most internally—*i.e.*, nearest to the tooth papilla. (9) The enamel cap is limited both externally and internally by a *membrana propria*. (10) The enamel cap becomes divided into an inner and outer membrane by the transformation of the middle layer (8 *c*) into a transparent tissue. *The inner membrane* is composed of columnar cells, the enamel cells, in contact with the dentine; each is a long hexagonal prism, and is nucleated at its lower part. Outside the layers of enamel cells are one or more rows of small polyhedral cells, forming the stratum intermedium. *The outer membrane* is composed of several layers of epithelial cells. (11) *The enamel* is formed by the enamel cells of the inner membrane elongating at their distal extremities; the elongated portion is transformed directly into enamel. (12) The cells of the stratum intermedium are used for the regeneration of the enamel. (13) The cells of the outer epithelium produce the *enamel cuticle*. (14) *The cement* is formed from the tissue of the tooth sac in exactly the same way as sub-periosteal bone is developed. (15) During the stage of the primary enamel organ (1) a lateral process grows out from the epithelial cells, which represents the rudiment of the enamel organ of the permanent tooth (*sac of reserve*). (16) *The permanent* teeth are developed on exactly the same plan as the deciduous set. (Klein.)

(C) SALIVARY GLANDS.

Preparation.—Sections of the submaxillary gland of a cat or dog, and of the parotid of a rabbit or dog, should be made in various directions after hardening in chromic acid and spirit.

Structure.—As of compound tubular glands, in which a

lobule is made up of convolutions of a main division of a duct bound together with connective tissue. The convoluted parts are lined by and almost filled with a single layer of columnar cells (*salivary cells*) enclosing a nucleus. The granular appearance which is frequently seen in the salivary cells is due to the very dense network of fibrils which they contain. These cells, when isolated, are not unfrequently found to be branched. The basement membrane of the tubes consists of branched and flattened cells, and between it and the salivary cells are found, here and there, (not in the parotid,) granular semilunar bodies, *the semilunes of Heidenhain.* The smallest divisions of the ducts have a relatively small lumen, and are lined near the convolutions with flattened epithelium, and then with nucleated columnar cells which present a longitudinal striation. The larger ducts acquire an outside coating of connective tissue, and are lined with a single layer of columnar epithelium, containing an intracellular network of fibres arranged longitudinally. In the walls of the largest ducts are unstriped muscular fibres.

The capsule consists of fibrous tissue, which sends septa into the substance of the gland, supporting the blood-vessels, lymphatics, nerves, and ganglia.

The salivary glands are divided into (*a*) *Mucous glands* in which the alveoli are large, and the cells are (1) mucous cells, transparent and columnar, with their pointed extremities applied to the membrana propria; the cells are imbricated; the nucleus is much compressed, and is near the membrana propria. (2) The semilunes of Heidenhain, or the crescents of Giannuzzi, semilunar groups of cells situated here and there between the mucous cells and the membrana propria. The cells are small and polyhedral, with a spherical nucleus. The submaxillary and orbital glands of the dog, and the sublingual gland of man are of the mucous type.

(*b*) *Serous glands;* in which the lumen of each alveolus is small, and the epithelium consists of a single layer of short columnar cells, each with a spherical nucleus situated at the periphery of the cell. The parotid gland and the greater part of the submaxillary gland of man and the guinea-pig, as well as the submaxillary and orbital glands of the rabbit, are serous glands.

(*c*) *Muco-salivary glands*, such as the submaxillary gland of man and the guinea-pig, are formed by the mixture of the mucous and serous types of glands.

(D) TONSILS.

Preparation.—Sections made from a tonsil which has remained for a week in ¼% solution of chromic acid, and subsequently in spirit, should be stained with logwood, and mounted in Canada balsam. An enlarged tonsil which has been removed from a child will answer the purpose.

Structure.—A tonsil consists of an elevation of the mucous membrane presenting upon its surface fifteen orifices leading into crypts or recesses, in the walls of which are placed nodules of lymphoid tissue. These nodules are enveloped in a less dense lymphoid or adenoid tissue which reaches to the mucous surface. The mucous surface is usually covered with squamous epithelium, and may present rudimentary papillæ which are then formed of adenoid tissue. The tonsil is bounded by a fibrous capsule. Into the crypts open a number of ducts of mucous glands.

(E) ŒSOPHAGUS.

Preparation.—Small pieces of the œsophagus (both of the upper and lower parts) of a dog should be hardened in chromic acid and spirit. The mucous glands are best seen in sections from the lower part of the œsophagus.

Structure.—Of three coats: 1. An *external* or *muscular coat* consists of two layers, longitudinal and circular, the former or external layer at the commencement being disposed in three fasciculi, one in front and one on each side. At the upper end of the œsophagus, the muscular coat is red, and consists of striated muscle; lower down it becomes paler, and the fibres are mostly unstriated. 2. A *submucous coat* consists of areolar tissue, and contains mucous glands (tubular), whose ducts pass inwards to open on the mucous membrane. 3. A *mucous coat* which is firm and wrinkled, provided with minute papillæ, and covered with a thick layer of stratified epithelium. It is separated from the submucous coat by a layer of unstriated muscular fibres, longitudinally arranged, which is partially imperfect as a layer above, but complete below (*muscularis mucosæ*).

The *arteries* situated in the submucous tissue give off branches which form a network of capillaries in the upper part of the

mucous coat; from this network loops are given off to supply the papillæ.

(F) STOMACH.

Preparation.—The stomach of a cat or dog may be used. After removal from the recently killed animal, the organ should be turned inside out, and washed with a gentle stream of weak bichromate of potash or spirit. Pieces of the mucous membrane from different parts should be snipped off with a sharp pair of scissors, and placed in strong alcohol. Other pieces should be cut off to show all the coats; these may be hardened in weak chromic acid, or in chromic acid and spirit mixture.

Sections must be cut both vertical and parallel to the surface at different depths.

To demonstrate the structure of the glands of the mucous membrane, some sections from each part should be stained in logwood, and others in anilin blue ·5% solution, as the anilin will stain the peptic cells very deeply, and so differentiate them from the cubical central cells. This is especially evident in horizontal sections of the peptic glands. The anilin tinted specimens must be passed through slightly acidulated water, as usual in anilin staining, before they are placed in spirit.

Structure.—The stomach is made up of four coats:—

1. The *mucous or internal coat* is smooth, soft, and pulpy, pink, becoming grey soon after death. Thickest at pylorus, thinnest at the great curve. Loosely connected with the muscular coat by means of the submucous, and so presenting temporary ridges (rugæ) when the organ is contracted. It consists almost entirely of small tubular glands, arranged close to and parallel with each other, varying in diameter from $\frac{1}{150}$ to $\frac{1}{100}$ of an inch, and in length from $\frac{1}{60}$ to $\frac{1}{30}$ of an inch, lined to a variable extent by columnar epithelium, which also covers the whole of the mucous membrane.

The tubular glands are for the most part simple, except near the pylorus, where they become larger, longer, and branched. The glands consist of a basement membrane formed of branched stellate cells joined edge to edge, and sending processes on the one hand to join the retiform tissue of the mucous membrane, and on the other to support the gland cells. The glands are of two kinds, differing chiefly in the character of the cells and

of their secretion. The one, the so-called "*mucous or pyloric glands*," are often branched, are confined to the pylorus, and are lined throughout by columnar epithelium, but towards the "fundus," or closed extremity of the gland, the cells tend to become cubical. The other, *the peptic glands*, are distributed throughout the whole of the mucous membrane, except at the pylorus, but are most typical perhaps towards the cardia; they are less often branched, but two glands generally open into one duct, which occupies a third of the whole length of the gland. The lower end, or fundus, is somewhat dilated, and sometimes slightly curved. The duct is lined with columnar epithelium, its middle third contains two distinct kinds of cells; outside, large granular cells with small nuclei, bulging out the basement membrane, and making irregular the outline of the tubes, called *peptic or parietal cells;** and inside, a layer of smaller finely granular cubical cells, *central cells*, which bound a small lumen. In the lower third, or fundus, the parietal cells do not form a continuous layer, but occur here and there irregularly; the remainder of the tube is filled with cubical central cells, which leave a very small lumen unoccupied. The cubical cells closely resemble those lining the fundus of the pyloric glands.

Between and beneath the glands is a quantity of delicate connective tissue, forming the mucous membrane proper, which here and there is collected into small masses somewhat resembling the solitary follicles of the intestine. A double layer (circular and longitudinal) of unstriated muscle (*muscularis mucosæ*) separates the mucous membrane from the submucous coat.

2. The *submucous coat* consists of areolar tissue with some fat, together with blood-vessels and lymphatics; small nerve ganglia and fibres are also found in it.

3. The *muscular coat* consists of three layers of unstriated fibres, externally of longitudinal, then of circular, and internally of oblique fibres; the circular layer is the only complete one. Between the layers may be fouud *plexuses of nerves.*

4. The *serous coat* is the peritoneal covering of the organ.

The arteries, after penetrating the muscularis mucosæ, break up into capillaries in the mucous membrane, which form a more

* It may be as well to mention that the so-called "peptic cells" are no longer thought to secrete the pepsin.

or less elongated meshwork around the glands. Near the surface the meshwork is very dense, and forms a well-marked superficial layer beneath the epithelium.

(G) SMALL INTESTINE.

Preparation.—To study epithelium in the fresh state, a scraping from the mucous membrane of the intestine of a recently killed animal may be teased and mounted in saline solution.

For the purpose of studying the relations of the various structures in the mucous membrane—*e.g.*, villi, Brunner's and Lieberkühn's glands, Peyer's patches, etc.—small pieces from each part of the intestine of the cat, dog, or rabbit should be hardened in weak chromic acid or chromic acid and spirit mixture.

To demonstrate the large lymphatic sinus surrounding the follicles comprising a Peyer's patch, the ileum is used, and the sinus is injected with Berlin blue by the puncture method,* whilst ·5% solution of silver nitrate will demonstrate the endothelial lining of the vessel.

To demonstrate the absoprtion of fat by the villi, a part of the intestine of an animal recently fed on fatty food should be ligatured and placed in Müller's fluid; in about a week's time pieces may be placed in osmic acid for twenty-four hours, and should afterwards be replaced in the solution.

For preparation of Meissner's plexus and the ganglia of Auerbach, see p. 68.

Structure.—There are four coats: 1. The *mucous coat possesses* (*a*) *valvulæ conniventes*, which are permanent folds or crescentic projections running transversely to the axis of the intestine, and containing the submucous coat. They first appear in the duodenum, not far from the pylorus; are largest in the duodenum and upper half of the jejunum, and then gradually become smaller, until they disappear about the middle of the ileum. (*b*) *Villi* are small processes, closely set on every part of the small intestine, over the valvulæ conniventes, as well as between them. They are conical and flattened in form, sometimes cylindrical, or with the free end clubbed. Largest in duodenum and jejunum, in length varying from one-fourth to one-third of a line; smaller, shorter, and fewer in ileum. They consist of pro-

* See Part III.

jections of the mucous membrane, being covered with columnar epithelium, enclosing blood-vessels, lymphatics, and muscularis mucosæ, bound together by fine retiform tissue, which also forms the basement membrane. (*c*) *Crypts of Lieberkühn* are very numerous small tubular glands existing everywhere in the small intestine; they are lined with columnar epithelium. (*d*) *Brunner's glands* are small compound tubular glands found in the duodenum, lying in the submucous coat, the ducts of which pass through the mucous coat. (*e*) *Peyer's glands*, or lymphatic follicular glands, which occur either solitary or collected ("agminated") into oblong patches. When solitary, they are found everywhere in the small intestine, both between and upon the valvulæ conniventes; when agminated, they occur in the ileum, especially at its lower part, in its long axis opposite the attachment of the mesentery. (2) The *submucuous coat* resembles that of the stomach, as does also (3) The *muscular coat*, but this has no oblique fibres. (4) The *serous coat* of the duodenum is partially incomplete.

The arteries passing through the muscularis mucosæ give off numerous capillaries which form a network around the crypts of Lieberkühn; the artery which passes into the villus generally ascends to the apex, and then breaks up into a dense plexus of capillaries, which spread over the apex and base. The capillaries are always situated in the periphery next the epithelium. There are generally one or two veins developed from the capillaries of the villus.

(H) LARGE INTESTINE.

Structure.—As of the small intestine, with the following differences:—

1. *Mucous coat* has neither true villi nor valvulæ conniventes, and its crypts of Lieberkühn are longer, more numerous, and are placed more closely together. The lymphoid follicles are always solitary.

Naked-eye differences.

2. *Muscular coat*—in the colon and cæcum the longitudinal layer is collected into three flat bands.

3. *Serous coat* of the colon and upper part of rectum is developed into small projections containing fat (appendices epiploicæ). It is incomplete in some parts.

The lymphatics of each villus consists of a single central vessel, or of two such vessels anastomosing with each other.

(I) PANCREAS.

Preparation.—The pancreas of a recently fed dog should be taken, cut into pieces about the size of a small hazel nut, and placed at once in absolute alcohol to harden. For the sake of comparison, another pancreas from a fasting dog should be treated in a similar manner. Sections should be stained and prepared in the usual way. Unless hardened in alcohol, the gland is very likely to become useless (from self-digestion ?) for microscopic purposes.

Structure.—The capsule and septa, as well as the blood-vessels and lymphatics, are arranged as in the salivary glands; the gland is, however, looser and softer, and the lobes and lobules are less compactly arranged.

The larger ducts possess a very distinct lumen, and a membrana propria lined with columnar epithelium cells which are longitudinally striated, but are shorter than those found in the ducts of the salivary glands. *In the smaller ducts* the epithelium is short and the lumen is smaller. The *intermediary ducts* opening into the alveoli possess a distinct lumen, with a membrana propria lined with a single layer of flattened elongated cells. *The alveoli* are branched and convoluted tubes, with a membrana propria and a single layer of columnar cells. The cells consist of an outer part nearest the membrana propria, which is homogeneous, and stains the more deeply; and an inner, more granular, and less readily stained portion. The alveoli do not, however, contain the semilunar granular bodies ("semilunes of Heidenhain"), and have no distinct lumen, its place being taken by fusiform or branched cells.

(J) LIVER.

Preparation.—Small portions of the fresh liver of a pig, rabbit, or puppy should be steeped for four or five days in a 2% solution of potassium bichromate, and then for one or two days in methylated spirit. Sections should afterwards be cut and treated as usual. It is as well also to mount sections of liver which has been injected through the portal vein with 2% solution of Berlin blue, and then hardened in spirit.

Structure.—It has a serous and fibrous coat. The former is absent from the posterior border, and from the portal fissure, where the latter, which elsewhere is thin, is most developed. A strong sheath of areolar tissue ("*Glisson's capsule*") surrounds the vessels of the organ as they ramify in it, and, at the transverse fissure, becomes continuous with its fibrous coat. *The liver substance* proper consists of lobules, which are closely packed polyhedral masses more or less distinct, arranged around the sides of the branches (sublobular) of the hepatic veins, and connected to them by minute veins which begin in the centre of the lobules (*intralobular veins*). *Each lobule* consists of a mass of compressed spheroidal or polyhedral nucleated and nucleolated cells from $\frac{1}{1000}$th to $\frac{1}{70}$th of an inch in diameter, often containing oil globules. Surrounding the lobules is a variable amount of fine connective tissue, in which is contained a minute branch (*interlobular*) of the portal vein, a branch of the hepatic artery and of the hepatic duct, together with minute lymphatic vessels covering them. The lobules are distinct when the interlobular tissue forms complete septa around them; if the septa are incomplete, the lobules become confluent. Fine fibrous tissue surrounds the interlobular vein, and a delicate supporting network of flattened branched corpuscles exists within the lobule between the cells and the blood capillaries. Between the columns of the cells run the radicals of the hepatic vein which open into the intralobular vein, and between the cells begin the radicals of the hepatic duct. Whether these radicals or bile capillaries have a definite membrana propria is undetermined. The interlobular *bile ducts* are endothelial tubes with a large lumen, lined with columnar epithelium. The larger ducts are surrounded with circular unstriped muscle cells, and have a distinct mucous membrane of loose connective tissue lined with columnar epithelium, and containing mucous tubular glands. The *lymphatics* of the lobule originate in the spaces around the capillaries of the lobules. The branches of the *hepatic artery* run between the lobules with the interlobular veins; in parts they surround the veins as a plexus; the arterial branches frequently anastomose with each other, and give off capillaries to supply the surrounding connective tissue and vessels, the bile ducts receiving numerous branches. The ultimate capillaries enter the lobules, where they form a plexus. The blood from

the artery is carried away by a special set of veins which open into the interlobular veins, none of it passes into the intralobular veins.

THE GALL BLADDER

is similar in structure to the large hepatic ducts, but the *mucous membrane* is thicker, and is thrown into folds and villous projections. *The muscular coat* also is thicker, and is surrounded by *connective tissue* and an outer layer of *peritoneum.*

RESPIRATORY TRACT.

EPIGLOTTIS.

Preparation.—The human epiglottis, removed as soon as possible after death, should be used. It should be cut into small pieces, and placed in the chromic acid and spirit mixture until sufficiently hardened. Transverse and vertical sections should be well stained in hæmatoxylin, and mounted in the ordinary way.

Structure.—The epiglottis consists of a supporting cartilage of the elastic variety, enclosed in a fibrous perichondrium, and covered on both sides with mucous membrane. The *anterior surface, i.e.*, the one towards the tongue, is covered by mucous membrane which hardly differs from that of the pharynx. This membrane consists of fibrous tissue, elevated towards the surface, in the form of rudimentary papillæ, and covered with several layers of squamous epithelium. In it ramify the capillary blood-vessels, and in its meshes are a large number of lymphatic channels. Under the mucous membrane in the less dense fibrous tissue, or submucosa, are a number of tubular mucous glands. The *posterior or laryngeal surface* is covered by a mucous membrane which is similar in structure to the above, but the epithelial coat is thinner, the strata of cells being less numerous. The papillæ are fewer and less distinct. The proper substance of the mucous membrane appears to be in great part adenoid tissue, which here and there is collected into

distinct masses. The glands of the posterior surface are smaller, but more numerous, than those on the anterior surface. In many places, the glands, which are situated nearest to the perichondrium, are directly continuous through apertures in the cartilage with those on the other side, and not unfrequently the ducts of the glands from one side of the cartilage pass through and open on the mucous surface of the other. Occasionally the epithelium of the posterior surface is columnar.

THE LARYNX.

Preparation.—As of the epiglottis.

Structure.—The structure of the larynx closely resembles that of the epiglottis on the one hand, and that of the trachea on the other. *The framework* is hyaline cartilage enclosed in a fibrous sheath, and covered internally by a mucous membrane. The epithelium covering this membrane is columnar ciliated, of which some of the cells are goblet cells, except over the upper part of the false vocal cords, the arytenoid cartilages, the true vocal cords, and immediately below, where it is stratified squamous. There is a distinct basement membrane under the epithelium. *The mucosa* is a dense fibrous tissue containing a large quantity of adenoid tissue; it is here and there separated from the submucosa, in which the glands are contained, by a thin layer of elastic fibres. *The submucosa* is scanty near the true vocal cords, and contains no glands; elsewhere it is distinct, and in it, as is generally the case with mucous membranes, the large vessels and nerves split up for the supply of the superficial structures. Taste goblets are found in the epithelium of the posterior surface of the epiglottis, in that of the ary-epiglottidean folds, of the inner surface of the arytenoid cartilage, and also on the true vocal cords. (Schofield, Davis.)

THE TRACHEA AND LUNG.

Preparation.—Distend the lungs of a recently killed rabbit or cat, through the trachea, with $\frac{1}{8}\%$ solution of chromic acid, tie up the trachea, and immerse it in a large quantity of chromic acid of similar strength. Change the solution for one of a $\frac{1}{4}\%$ in two days; in a week cut in pieces, and remove to methylated spirit.

Before cutting sections it is necessary that the embedding

mass shall have thoroughly penetrated into and filled up the interstices of the tissue, and so it is best to place the piece of lung to be embedded in the wax mass when it is quite hot. In some cases it is as well to stain the lung with logwood, and pass it through alcohol and oil of cloves before embedding. Unless the interstices are filled up, it is almost impossible to cut thin sections.

To free the cut sections from wax, pass them through oil of turpentine before putting them into oil of cloves.

A better method is to soak the lung in gum, and then to cut sections of it by means of the freezing microtome; afterwards removing the gum by immersion in warm water.

Thin sections of lung injected through the pulmonary artery with Berlin blue, and through the trachea with ¼% solution of silver nitrate, should be made and treated in the usual manner. Sections of trachea should also be made.

Structure.—(1) *Of trachea.* (*a*) *An elastic framework* of incomplete rings or hoops of hyaline cartilage, 16 to 20 in number; each presents a curve of rather more than ⅔ of a circle. These rings are held together by a strong fibrous membrane, more or less elastic, which not only occupies the interval between them, but is prolonged over their outer and inner surfaces; behind, where the cartilage is incomplete, the fibrous membrane is strengthened by a continuous layer of unstriated muscle, principally transverse. (*b*) *A submucous coat* of areolar tissue and fat, containing also, immediately beneath the mucous membrane, longitudinal fibres of elastic tissue, collected for the most part into bundles. Tubular mucous glands are found in this coat, and also upon and beneath it. (*c*) *A mucous membrane* containing a large amount of lymphoid tissue, under the epithelium a basement membrane of flattened cells, which send up processes to the epithelium. In the deeper parts are many elastic fibres. On the surface are several layers of epithelium, of which the more superficial are columnar and ciliated, often branched below to join the connective-tissue corpuscles. Between the branched ends of these cells are smaller elongated cells, prolonged upwards towards the surface, and downwards to the basement membrane. Beneath these are one or more layers of irregularly shaped cells.

(2) *Of bronchi*, as of trachea.

(3) *Of lung.* The tissue is made up of lobules attached to the minute divisions of the air-tubes, by which they are held together, as well as by blood-vessels and interlobular tissue. The lobules, although adherent, are quite distinct; the structure of each represents that of the entire lung, and consists of a minute *air-tube* with terminating *air-cells*, lined with tesselated *epithelium*, together with the pulmonary and bronchial blood-vessels, lymphatics, nerves, and areolar tissue. The principal divisions of the bronchi divide, generally dichotomously, into branches running in all directions, which never anastomose, but terminate separately in the lobules; within the lobules each bronchial tube finally ends in small recesses (air-cells, alveoli, or vesicles) having previously lost its cylindrical form, from being beset with similar air-vesicles on all sides; in this condition the tube becomes what is called an *infundibulum*. The structure of the air-tubes gradually changes as they become smaller. The cartilages become irregularly shaped plates and rings of different sizes, scattered over the sides of the tubes, gradually becoming fewer, and finally disappearing before the infundibulum is reached. The fibrous coat extends to the smallest tubes, by degrees becoming simply areolar. The mucous membrane becomes thinner, but retains its former epithelium, the cells becoming very short columnar in the smallest bronchi. The longitudinal elastic bundles are traceable into the smallest tubes. The muscular fibres ultimately form a continuous circular layer inside the cartilaginous plates. The walls of the infundibula consist of (*a*) unstriped muscle arranged circularly; (*b*) a network of elastic fibres; (*c*) fibrous tissue and connective-tissue cells; (*d*) a dense meshwork of capillary blood-vessels; (*e*) small polyhedral cells and large flattened cell plates. These plates vary in shape and size according to the amount of distension of the air-vesicles; they are best seen in lungs stained with nitrate of silver. (*f*) Between the cell plates *pseudo-stomata* may be found; *i.e.*, larger or smaller circular angular openings similar to the stomata found in serous membranes; they lead into the lymph-canalicular system of the alveolar wall.

The blood-vessels constitute a dense capillary plexus upon the alveolar septa: in the contracted lung the capillaries are very sinuous and close together, whilst in the distended lung they are straighter and further apart. Near the pleura and bronchi the

capillaries anastomose with the capillaries of the bronchial artery. The larger arterial and venous branches are situated in the interlobular connective tissue, which is continuous with their outer coat. *The lymphatics* are arranged in three systems. (*a*) The subpleural lymphatics, forming a dense plexus whose meshes mostly correspond with the outlines of the alveoli. (*b*) The perivascular lymphatics, whose vessels accompany the branches of the pulmonary artery and vein. (*c*) The peribronchial lymphatics remaining in the outer coat of the bronchi, and anastomosing freely with the perivascular lymphatics.

SKIN AND APPENDAGES.

Preparation.—Small pieces of skin from various parts—*e.g.*, from palm of hand, fingers, or toes, scalp, scrotum, and general surface, should be hardened in equal parts of chromic acid, ½%, and of methylated spirit, for a week, changing the liquid on the second, fourth, and seventh days, and then removing to spirit until required. Sections may be made in various directions (cutting towards the epidermis is the easiest way), stained, prepared, and mounted in the usual manner.

Double staining, with picrocarmin as well as with logwood, is recommended.

Injected specimens of skin may be prepared by injecting 2% Berlin blue solution into the main artery of a limb of a dog, or one of the upper extremities of a fœtus.

Structure.—The skin consists of two parts :—

1. *Epidermis, or external skin*, is made up of several more or less distinct layers. (*a*) The most superficial horny layer (stratum corneum) varies in thickness, is composed of layers of flattened epithelium, which show nuclei only after treatment with softening reagents, *e.g.*, caustic potash. (*b*) The next layer (stratum lucidum) is generally homogeneous and thin ; it is composed of closely packed scales. (*c*) A layer of granular cells (stratum granulosum), flat, spindle-shaped, and nucleated, which stain deeply in logwood. (*d*) Finally, the Malpighian layer (rete Malpighii, or rete mucosum), consisting of stratified epithelium,

membrane of endothelial cells. To the outside of the follicles thin bundles of unstriped muscular fibre are attached, and into the follicle open the ducts of sebaceous (simple tubular) glands, generally one on each side.

NAILS.

Preparation.—Sections may be cut of the end of a finger after hardening.

Structure.—Nails are composed of flattened epithelial scales, and are equivalent to the superficial or horny layer of the epidermis. The deeper layers of the nail are softer than the more superficial. Underneath the nail are highly vascular papillæ (bed or matrix). Posteriorly it is received into a groove in the skin (root). The growth of the nail is effected by constant additions of cells to the root and under surface, so that it grows in length and in thickness at the same time.

GENITO-URINARY ORGANS.

KIDNEY.

Preparation.—Hardened in the same way as the liver. Sections should be made in various directions. Sections of injected kidney should also be prepared. The best injecting material is either carmine-gelatin or Berlin blue.

Structure.—There is a distinct fibro-areolar coat, thin, firm, smooth, and easily detached. The proper substance of the organ is divided into three regions, *the cortical region*, *the boundary layer*, and *the papillary region*. On a vertical section through the kidney, the cortex is that lighter part nearest the capsule, whilst the redder portion is the medulla, which is seen to be made up of a number of pyramidal portions, each papilla converging to the interior and towards branches (*calices*) of the dilated portion of the main duct or *pelvis* of the kidney. Each calyx encloses two or three papillæ. The part of the base of the pyramid towards the cortex, between it and the papillary portion, is called, as above mentioned, *the boundary layer*. The pyramid

itself is called the *pyramid of Malpighi*. The papillary portion is distinctly and vertically striated from the vertical direction of both tubules and blood-vessels, of which the kidney is principally made up. The boundary layer is also striated for a similar reason; but the cortex, although containing vertical columns, from the arrangement of some of the tubules (*medullary rays*), no longer contains the vessels arranged in vertical directions, nor are all the tubules straight, but convoluted, forming the *labyrinth*. From the medullary rays diminishing in thickness from the boundary layer outwards towards the capsule, each presents a triangle, with its base at the boundary layer. These triangles are called the *pyramids of Ferrein*. The cortical substance separates the pyramids from each other, and encloses them everywhere except at the papillæ; one layer of it, situated immediately beneath the capsule, forms the most superficial part of the organ. The portion of the cortical substance intervening between two pyramids is known as the *columns of Bertini*. The papillæ are studded with minute openings leading into tubes (*tubuli uriniferi*), through which the urine passes out into a primary division (*infundibulum*) of the pelvis, or dilated part of the duct (*ureter*) of the kidney. The tubes of the pyramids, as they pass up, divide again and again at very acute angles, until they arrive at the cortical layer, and then become convoluted. Each tube begins in a spherical dilatation (*Malpighian capsule*), enclosing a tuft of minute vessels (*Malpighian tuft*). Arising thus in the cortex, a tube is at first convoluted, and consists of a basement membrane lined and almost filled with granular epithelium; afterwards becoming smaller, it passes straight down the pyramid towards the papilla, and returns again, forming a *looped tube of Henle* lined with squamous epithelium, then again becomes convoluted, and finally joins a branch of a straight tube of the pyramid (*collecting tube*). The collecting tubes are lined with columnar epithelium, and joining together form the excretory tubes or ducts of Bellini, which open at the papilla.

According to recent observations, a renal tubule is said to be made up of the following sections (Klein):—

(1) The *Malpighian capsule*, lined with squamous epithelium.

(2) The *neck*, a constricted portion joining the capsule, and lined in the same way.

(3) A portion enlarged and convoluted, called the *proximal*

convoluted tube, lined with polyhedral or short columnar cells, with a lumen of about one-third of the diameter of the tube. The cells are vertically striated.

(4) *The spiral tube*, which passes downwards, and is composed of the same structures as (3).

(5) The constricted portion, called *the descending limb* of the loop of Henle.

(6) *The loop of Henle*, lined with squamous epithelium, as is (5).

(7) *The ascending limb*, which becomes rather suddenly enlarged ; this portion is lined with striated epithelium.

(8) *The spiral portion of ascending loop* is again somewhat constricted.

(9) The ascending loop again becomes narrower, but is straight.

(10) *The irregular tubule* has a very irregular and angular outline, sometimes being three or four times as thick as at others ; this is due to the irregularity in the size of the contained epithelium. The cells are striated, angular, and imbricated.

(11) *The intercalated section* (Schweigger-Seidel), or the distal convoluted tube, is similar to (3).

(12) and (13) Curved collecting tubes are thin tubes, lined with polyhedral cells, or spindle-shaped and flattened.

(14) The straight collecting tube, which passes into the boundary layer, and enters—

(15) The large collecting tube, or *tube of Bellini.*

(16) The tube of Bellini, having anastomosed with similar tubes, forms the main tube of the pyramid which opens into the calyx of the pelvis, with a "*mouth*" at the apex of the papilla.

Blood is supplied to the kidney by the renal artery, which divides into branches which lie between the cortex and the boundary region ; smaller vessels pass up and enter the cortex, and pass down to supply the medulla. The vessels of the cortex pass up in the labyrinth between the medullary rays (interlobular) and give off transverse branches, the afferent vessels of the Malpighian tufts ; these break up within the capsule into convoluted capillaries, re-uniting into the efferent veins ; these again break up into capillaries around the convoluted tubes, to be afterwards collected into small branches of the renal vein. The vessels of the medulla break up in the boundary region, and

send off straight vessels between the tubes of the papillary region (*vasa recta*); the vessels decrease in number towards the papilla, as most of them break up into capillaries around the tubules, which capillaries anastomose near the cortex with those of that region. The veins of the papillary region begin simply in the papilla, increasing in size and number as they pass upwards; join with the veins of the cortex to form the main branches of the renal vein which accompany the main branches of the renal artery, and lie between the cortex and medulla, as above mentioned.

A certain amount of interstitial connective tissue is found supporting the tubes and blood-vessels.

URETER.

Preparation.—Tie one end of the ureter, and distend it with chromic acid and spirit; leave for one day in the same mixture; then slit open, and leave in spirit for a week. The cells may be shown by hardening a piece of ureter in bichromate of potash 1% solution, staining deeply in logwood, and scraping the inside, teasing and mounting in glycerin.

Structure.—Consists of three coats :—(1) External *fibrous;* (2) middle of two layers (circular and longitudinal) of unstriped *muscular fibres;* (3) internal or *mucous*, lined by stratified epithelium, the upper cubical cells of which have their under surfaces hollowed out to receive the second layer of pear-shaped cells.

BLADDER.

Preparation.—As of the ureter. A double staining with eosin and logwood brings into view the differences in the form of the lining cells.

Structure.—Consists of four coats: (1) *Serous* or external —is incomplete, as it is only found at the upper and posterior parts. (2) *Muscular*, consisting of three layers more or less complete—viz., (*a*) external longitudinal, (*b*) circular, (*c*) internal longitudinal. (3) *Submucous* of connective tissue. (4) *Mucous*, lined with stratified epithelium, the upper layer being made up of polyhedral cells, with one, two, or three nuclei, presenting depressions with intervening ridges for the second layer of club-shaped cells; the next layer is made up of more fusiform cells.

PROSTATE.

Preparation.—¼% chromic acid for two days, followed by spirit.

Structure.—Small compound tubular glands imbedded in an abundance of muscular fibres and connective tissue. The glandular substance consists of numerous small saccules, opening into elongated ducts, which unite into a smaller number of excretory ducts. *The acini*, in the upper part of the gland, are small and hemispherical; whilst in the middle and lower parts the tubes are longer and more convoluted. The acini are of two kinds, (*a*) lined with a single layer of thin and long columnar cells, each with an oval nucleus in outer part of wall, (*b*) acini resembling the foregoing, but with a second layer of small cortical, polyhedral, or fusiform cells between the membrana porpria and the columnar cells. *The ducts* are lined by a layer of columnar cells, beneath which is a layer of small polyhedral cells. The tunica adventitia is formed of loose connective tissue containing fat. Large *vessels* pass into the interior of the organ, to form a broad, meshed, capillary system. *Nerve trunks* and numerous large ganglion cells surround the cortex. Pacinian bodies are also found in the substance of the prostate.

VAS DEFERENS.

Preparation.—By hardening in a 2% bichromate of potash solution for fourteen days, after which the tissue is placed in spirit.

Structure.—Like the vesiculæ seminales, of three coats: (1) *External*, of connective tissue, outside which longitudinal fibres of unstriated muscles are often seen. (2) *Muscular*, two longitudinal layers with an intermediate circular one. (3) *Mucous*, of connective tissue and elastic fibres; this layer is often thrown into three or four longitudinal ridges; the epithelium consists of columnar epithelium, ciliated only near the epididymis. (4) *The nerves* form a plexus in the tunica adventitia.

TESTES.

Preparation.—Place the testes, preferably of rat or cat, after making two or three cuts in it, in equal parts of chromic acid

½% and methylated spirit. Change three times in a week, and remove to spirit, or inject a 1% solution osmic acid into the tunica albuginea, then place in strong spirit for several days, and afterwards for two days in absolute alcohol previous to making sections. Stain with hæmatoxylin or carmine; prepare and mount as usual.

Structure.—(1) Outer coat of connective tissue, the *tunica albuginea*, from which radiate incomplete septa uniting into a thick wedge-shaped body, the corpus highmori. The testis is divided by these septa into lobes, each consisting of small and convoluted tubes, the tubuli seminiferi. (2) The *tubuli seminiferi* are composed of a basement membrane of flattened endothelial cells, a single row in small animals, more than one in large animals, within which are a number of cells not arranged in any definite order—the seminal cells. The *outer* form a single row. The tubuli seminiferi have a uniform diameter of $\frac{1}{150}$ to $\frac{1}{100}$ in.; they commence in free closed extremities or in anastomosing arches, and unite to form the vasa recta. In transverse section the seminal tubules are seen to have a narrow lumen surrounded by polygonal cells, of which the peripheral ones are arranged radially. In the interstitial connective tissue between the tubuli seminiferi are a number of connective-tissue corpuscles. (3) From the *seminal cells* the spermatozoa are developed. (4) The *vasa recta*, about twenty in number, are $\frac{1}{90}$ to $\frac{1}{70}$ in. in diameter. They possess very thin walls, and pass upwards and backwards to terminate in the rete vasculosum testis. (5) The *rete testis* is lined with pavement epithelium, and opens into twelve to twenty vasa efferentia, forming the coni vasculosi. (6) The *coni vasculosi* are $\frac{1}{60}$ in. in diameter, and open into the canal at the epididymis. (7) The *epididymis* and *vasa efferentia* contain plain muscular fibres; the lining cells are columnar and ciliated, elongated in the epididymis, shorter in the vasa efferentia. (8) Remove and draw *spermatozoa* from the fresh glands, (*a*) the head, (*b*) the middle portion, (*c*) the caudal extremity. (9) The *blood-vessels* surround the convoluted tubules with a long-meshed wide capillary plexus. (10) The *lymph passages* form an extensive canalicular system.

VESICULÆ SEMINALES.

Preparation.—Either in $\frac{1}{6}\%$ chromic acid for seven days, followed by spirit, or the hardening may be effected by spirit alone.

Structure.—(1) External *connective-tissue* coat. (2) Middle *muscular* coat of three layers, the internal of longitudinal fibres, middle of circular fibres, external of longitudinal fibres. (3) *Mucous* coat thrown into rugæ, the epithelium of cylindrical cells provided with striated borders, the deep layer being polyhedral. The mucous membrane contains a few muscular fibres. (4) *Ganglion cells* and *nerve plexuses* are numerous in the outer coat. (5) According to Leydig, a number of racemose *glands* are present.

THE PENIS.

Preparation.—The penis of a human fœtus should be used, if it can be obtained, otherwise that of the cat or dog must be employed. It should be injected from the abdominal aorta, after ligature of the iliac arteries, and should then be hardened in $\frac{1}{4}\%$ chromic acid for a fortnight. Sections should be made in various parts.

Structure.—(*a*) *The urethra* is (1) lined by stratified pavement *epithelium* in the lower part of the prostatic and membranous portions; in the upper half the epithelium is of the stratified transitional variety; in front of the bulb the epithelium becomes columnar, whilst the fossa navicularis is again lined with stratified pavement epithelium. (2) *The mucous membrane* consists chiefly of fibrous connective tissue, intermixed with which are many elastic fibres. It is surrounded by muscular tissue of the unstriped variety. In the membranous portion many large veins run amongst the bundles of muscular tissue. (3) Many *mucous glands* are present.

(*b*) *The corpora cavernosa* consist of (1) *a matrix*, chiefly of unstriped muscle fibres, intermixed with which is a little connective tissue and a few elastic fibres. The matrix is arranged in bundles, and separates (2) the very large *venous sinuses* which constitute the greater part of the substance of each corpus cavernosum. The sinuses anastomose with each other to form plexuses, and each is lined by a single layer of flattened endothelial plates. The arteries run in the muscular trabeculæ. The

corpus cavernosum urethræ consists of an inner portion or plexus of longitudinal veins, and of an outer or really cavernous portion identical in structure with that which has just been described. *The lymphatics* of the penis are very numerous. *The nerves* form a dense subepithelial plexus. *Cowper's glands* resemble the sublingual gland; they are large compound tubular mucous glands. (Klein.)

OVARY.

Preparation.—The ovaries are placed, with as little handling as possible, in a mixture of equal parts of spirit, and ½% chromic acid solution for two or three days, and afterwards in spirit. The sections are to be stained with hæmatoxylin or carmine.

Structure.—The ovary consists of an encapsuled stroma and embedded Graafian follicles. *The outer coat* consists of low columnar epithelium cells, beneath which is a firm layer of fibrous tissue. *The stroma* of fibrous tissue and elastic fibres containing blood-vessels, and in the deeper portion muscular fibres. The cortical portion contains a large number of closely set vesicles, $\frac{1}{100}$ inch in diameter. Each vesicle, or primordial ovum, is surrounded by a corona of small nucleated cells. Below this layer of vesicles are more advanced ova, the deepest being the most mature. *The Graafian follicle*, $\frac{1}{20}$ to $\frac{1}{8}$ inch in diameter, contains a ripe ovum; and is surrounded by fibrous tissue, and by the tunica vasculosa, more internally by the tunica granulosa, consisting of several layers of granular prismatic cells. In a thickened portion of the tunica granulosa (discus proligerus), the ovum is embedded on the inner surface and to one side of the Graafian follicle. The tunica granulosa is separated from the discus proligerus, except at their point of union, by a space containing a clear albuminous fluid. *The ovum*, $\frac{1}{150}$ inch in diameter, consists (*a*) of an external, firm, transparent membrane, which is finely striated radially (vitelline membrane, or zona pellucida), (*b*) of a mass of granular protoplasm (vitellus, or yolk), (*c*) of a small clear vesicle, $\frac{1}{700}$ inch in diameter (germinal vesicle), embedded in the vitellus, and which encloses (*d*) a dark granular spot (germinal spot, or macula germinativa), $\frac{1}{3000}$ inch. *The corpus luteum* is a Graafian follicle which has discharged its ovum; it is filled with a reddish-yellow mass of elongated cells, the colour being due to the formation

of pigment, which, however, is not derived from the slight hæmorrhage which takes place on the escape of the ovum.

UTERUS.

Preparation.—Distend the uterus of a cat or rabbit with a mixture of equal parts of ½% chromic acid solution and strong spirit through the vagina. Tie the openings into the organ, and remove to a bottle containing same mixture. Change the solution at the end of twenty-four hours, and lay open the uterus. Two days later remove to spirit. Stain in hæmatoxylin.

Structure.—(1) *External serous coat* derived from the peritoneum. (2) *Muscular coat* intermixed with fibro-areolar tissue, blood-vessels, lymphatics, and some veins. The muscle is arranged in three layers: (*a*) the external longitudinal, the weakest coat; (*b*) transverse fibres forming the strongest layers; (*c*) oblique fibres which become annular to form the sphincter uteri. The cells constituting the muscular layers are fusiform, with long tapering extremities; the nucleus is always single. (3) *The mucous membrane* is smooth in the fundus and body of the organ; it is raised into transverse folds in the upper portion of the cervix; and forms papillæ in the terminal portion of the cervix. It is lined with columnar ciliated epithelium. The glands are tubular, often spiral, sometimes slightly branched. They are found in the fundus and body, and are lined with ciliated epithelium. Small closed sacs (ovula Nabothi) are also distributed regularly over the mucous membrane. (4) *The blood-vessels* are large and numerous; *the lymphatics* form large plexuses in the peripheral layers of the pregnant uterus; *the nerves* are medullated and non-medullated, a few ganglion cells being also present.

FALLOPIAN TUBES.

Preparation.—As for the uterus.

Structure.—(1) *External serous coat*, rich in vessels and in connective tissue. (2) Longitudinal, and a thicker circular layer of unstriated *muscle*. (3) *Mucous membrane* thrown into longitudinal rugæ, and lined with columnar ciliated epithelium; no glands are present, and as yet no nerves have been detected. The mucous membrane contains a layer of muscularis mucosæ.

MAMMARY GLAND.

Preparation.—The gland in pieces is placed in a solution of equal parts of spirit and ½% chromic acid solution for two days, afterwards in weak and strong spirit. It is to be stained in hæmatoxylin. Double staining may also be used.

Structure.—The mammary gland consists of a number of individual racemose glands united by intervening areolar tissue. (1) *The lobes* thus formed have a considerable quantity of adipose tissue between them, whilst the blood-vessels and the small medullated nerves run in the connective-tissue stroma. (2) The racemose glands open by means of ducts, *the lactiferous ducts*, which unite together until fifteen to twenty excretory canals are formed, (3) *the galactophorous ducts*, which converge towards the nipple. Near the nipple the galactophorous ducts become dilated to form sinuses, but they undergo constriction again before opening to the exterior. (4) *The gland vesicles* consist of a membrana propria with flattened stellate cells, lined by low columnar epithelium. The vesicles are filled with fat globules; and if the oil be extracted by immersion of the gland in ether, casein remains behind. The terminal vesicles are at first simple, but as the gland developes they produce buds. (5) *The ducts* consist of areolar tissue with a circular and longitudinal layer of elastic fibres; they are lined with low cylindrical epithelium, which becomes flattened near the nipple. Near the nipple also, and beneath the areola, unstriated muscular fibres are found. (6) *The blood-vessels* form a dense capillary network around the alveoli, forming a continuous system for each lobule.

DUCTLESS GLANDS.

THYROID GLAND.

Preparation.—By immersion of the gland for twenty-four hours in a mixture of spirit and water, then in strong spirit, till the tissue is sufficiently hard. The thyroid gland may also be hardened by allowing it to remain for a month in Müller's fluid, or in ¼% chromic acid for a fortnight. Stain in logwood.

Structure.—(1) A thin transparent layer of dense areolar tissue, free from fat, containing elastic fibres. This *connective-tissue framework* traverses the interior of the organ in the form of strong trabeculæ; it encloses (2) rounded or oblong irregular cavities, *the vesicles*. The vesicles consist of a thin hyaline membrane lined by a single row of low cylindrical cells. The cavities of the vesicles are filled with a coagulable fluid, or more frequently with a colloidal substance. The colloidal substance increases with age, and the cavities appear to coalesce. (3) In the interstitial connective tissue is a round meshed *capillary plexus*, and (4) a large number of *lymphatics*. (5) *The nerves* adhere closely to the vessels.

THYMUS GLAND.

Preparation.—As for the thyroid gland, the concentric corpuscles may be teased out from the fresh gland in normal saline solution. Stain with carmine.

Structure.—(1) *A capsule* of thin areolar tissue which sends down processes dividing the gland into lobules. The outer surface of the organ is covered with a layer of flattened cells. (2) Each *lobe* is made up of a number of polyhedral lobules, connected by delicate areolar tissue, which are (3) in turn composed of small *follicles*. The follicles are composed of adenoid tissue or retiform tissue, the meshes of which are filled up with lymphoid corpuscles. The follicles are therefore comparable with the spleen, tonsils, lymphatic glands, and Peyer's patches. (4) Scattered in the adenoid tissue are the concentric *corpuscles of Hassall*, composed of deeply staining substance, with high refractive index. Of these there are two kinds—one,

the smaller, simple; the other, the larger, compound. (5) *The arteries* radiate from the centre of the gland. (6) *The lymphatics* are large. (7) *The nerves* are very minute.

PITUITARY BODY.

Preparation.—As for the thyroid gland.

Structure.—Of two lobes—*a small posterior one*, consisting of grey nerve tissue; *an anterior larger one*, resembling the thyroid in structure. *A canal*, lined with flattened or with ciliated epithelium, passes through the anterior lobe; it is connected with the infundibulum. *The gland spaces* are oval, nearly round at the periphery, spherical towards the centre of the organ; they are filled with granules and nucleated cells. The vesicles are enclosed by connective tissue, rich in capillaries.

PINEAL GLAND.

Preparation.—By hardening in alcohol, and by maceration in Müller's fluid; or, better still, by means of osmic acid.

Structure.—A central cavity lined with ciliated epithelium. The glandular substance is divisible into—(1) *An outer cortical layer*, analogous in structure to the pituitary body; and (2) *An inner central layer*, wholly nervous. The cortical layer consists of a number of closed follicles, containing (*a*) cells of variable shape, rounded, elongated, or stellate; (*b*) fusiform cells. There is also present a gritty matter, the acervulus cerebri, consisting of round particles aggregated into small masses. The central substance consists of white and grey matter. (3) *The blood-vessels* are small, and form a very delicate capillary plexus.

SUPRA-RENAL CAPSULE.

Preparation.—In bichromate of potash 2% for a fortnight, in Müller's fluid for a month, or in osmic acid four hours; in each case complete the hardening in spirit.

Structure.—(1) An outer *sheath of connective tissue*, which sends in prolongations, forming the framework of the gland. (2) *The cortical portion*, divided into (*a*) an external layer of closed vesicles, the zona glomerulosa. The vesicles contain a finely granular greyish substance, no fat globules, but generally

a few small cells. (*b*) A layer of cells arranged radially, the zona fasciculata. The substance of this layer is broken up into cylinders, each of which is surrounded by the connective-tissue framework. The cylinders thus produced are of three kinds—one containing an opaque, resistant, highly refracting mass (probably of a fatty nature); frequently a large number of nuclei are present; the individual cells can only be made out with difficulty. The second variety of cylinders is of a brownish colour, containing finely granular cells, in which are fat globules. The third variety consists of grey cylinders, containing a number of cells whose nuclei are filled with a large number of fat granules. (*c*) The third layer of the cortical portion is the zona reticularis. This layer is apparently formed by the breaking up of the cylinders, the elements being dispersed and isolated. The cells are finely granular, and have no deposit of fat in their interior; but in some specimens fat may be present, as well as certain large yellow granules, which may be called pigment granules. (3) *The medullary substance* consists of closed vesicles; of elements of the cortical substance; of numerous blood-vessels; and of an abundance of nervous elements. The cells are poor in fat, and occasionally branched; the nerves run through the cortical substance, and anastomose over the medullary portion.

Blood-vessels.—The cortical portion is supplied with a rich plexus of capillaries, whose meshes are polyhedral in the outer and middle zones, and more elongated in the zona fasciculata. The medulla is supplied with a very rich plexus of wide capillaries. In all parts the blood-vessels are embedded in the trabeculæ.

Lymph spaces and sinuses best seen between the cells of the zona fasciculata, but existing in other parts, occupy the intercellular spaces and lacunæ; the efferent lymphatics provided with valves lie in the capsule and in the connective tissue around the central veins. (Klein.)

THE LYMPHATIC SYSTEM.

I. LYMPHATIC VESSELS.

(A) Trunks, (B) Capillaries.

(A) **Preparation.**—Make sections of a thoracic duct which has been hardened in bichromate of potash, and subsequently in spirit, stain with logwood, and mount in Canada balsam.

Structure.—Lymphatic trunks, such as the thoracic duct and the lymphatics leading to the mesenteric glands, have nearly the same structure as veins, and like them consist of three coats. They are provided with valves, especially at their subdivisions. The endothelial cells lining them are elongated.

(B) **Preparation.**—To demonstrate the structure of lymphatic capillaries, the epithelium covering the central tendon of the diaphragm of a rabbit or guinea-pig must be pencilled off with a camel's-hair brush, stained with nitrate of silver, and mounted in glycerin.

Structure.—Lymphatic capillaries consist of a single layer of sinuous endothelial cells, united together by intercellular substance so as to form a membrane.

II. LYMPHATIC GLANDS.

Preparation.—Thin sections of a lymphatic gland which has been previously hardened in Müller's fluid, or in bichromate of potash, must be stained with logwood, and shaken in a test tube half full of water for thirty minutes or more. They are then to be mounted in balsam in the ordinary manner.

Structure.—Each lymphatic gland is surrounded by a *capsule*, which consists of connective tissue intermingled with unstriped muscular fibres. From the capsule are given off a number of *trabeculæ*, which give support to the blood-vessels, and pass into the interior of the gland, so as to divide it into a number of compartments or alveoli, which contain the adenoid tissue or proper tissue of the gland. The *adenoid tissue* is arranged in the form of follicles in the cortex, and of rounded cords in the medulla. Between the walls of the alveoli and the proper tissue of the gland are a number of spaces lined by

endothelium—the so-called *sinuses* of a lymphatic gland. These sinuses are continuous on the one hand with the afferent vessels, and on the other with the efferent vessels.

III. THE SPLEEN.

Preparation.—Small pieces of fresh spleen are hardened in 2% solution of bichromate of potash, and subsequently in spirit, till they are fit for making sections. Injected specimens should also be examined.

Structure.—The spleen possesses two coats, a serous and a fibrous. The *serous coat* is derived from the peritoneum, and covers the organ almost completely. The *fibrous coat*, or tunica propria, is composed of connective tissue, which in some animals is intermingled with a large proportion of unstriped muscular fibres. From its inner surface processes or trabeculæ pass into the interior of the organ, and interlace freely with with each other, so as to form the *trabecular framework* of the spleen. At the hilus of the spleen the capsule is prolonged with the blood-vessels for which it forms sheaths, which become connected with the trabeculæ above described. The interstices between these trabeculæ contain the proper tissue of the spleen, or *spleen pulp*. The spleen pulp is composed of an adenoid reticulum, forming meshes, with endothelial plates attached; into these meshes the small arteries pour their blood, and with these meshes, by their widening out, and by the arrangement of the endothelial plates into a distinct lining, the veins are continuous. The spleen pulp contains red blood corpuscles in all states of decay and renovation, a large number of colourless corpuscles and blood pigment. Small arteries pass off almost at right angles from the branches within the trabeculæ, into the spleen pulp, and exchange their outer coat of connective tissue for one of adenoid tissue. This adenoid sheath, which takes the place as it were of the lymphatic vessels which surround the arteries whilst they lie in the trabeculæ, forms cords of adenoid tissue, with arteries occupying some position, not necessarily the central: the cords are not everywhere of the same diameter, increasing here, diminishing there; and on section appear as more or less circular masses of adenoid tissue, highly vascular when injected. To these masses seen on section the term *Malpighian corpuscles* has been applied.

THE EAR.

Preparation.—Remove the lower jaw from a recently killed guinea-pig, in order to expose the auditory bulla. Carefully break open the bulla after removing the soft parts from it, and look for the cochlea. Remove the cochlea by chipping away the surrounding bone, and immerse it at once in Müller's fluid. Ten days later transfer to a saturated solution of picric acid, until the bone is sufficiently softened to be cut with ease. Place it then in weak spirit, which should be changed as often as it becomes yellow from the picric acid. Finally, put the cochlea into absolute alcohol for twenty-four hours before cutting it. The preparation should be embedded in such a way that longitudinal sections can be made. Stain in hæmatoxylin. Cutting with the microtome is not admissible.

Structure.—*The cochlea* is a gradually tapering spiral tube, winding round a central column, *the modiolus*. It is divided along its whole extent by a spiral lamina, which projects from the modiolus, into two main portions—the scala tympani and the scala vestibuli. *The spiral lamina* is partially osseous and partially membranous. The membranous portion, *the basilar membrane*, is connected to the outer wall of the cochlea by its union with the *spiral ligament*, which is a projection inwards of the periosteum and subperiosteal tissue of the cochlea. *The scala vestibuli* is subdivided into scala vestibuli proper, and *ductus cochleæ*, by the *membrane of Reissner*, which passes from the spiral lamina to join the lining periosteum. *The membrane of Reissner* is composed of a delicate membrana propria, continuous with the periosteum, covering the scala vestibuli. It is lined with a layer of flattened endothelium on the face turned towards the scala vestibuli; whilst that bounding the ductus cochleæ is provided with a single layer of polyhedral cells. *The periosteum* consists of ordinary connective tissue, thickened here and there by retiform tissue. *The spiral ligament*, to which the basilar membrane is attached, consists of periosteum thickened by the retiform tissue, the cells being elongated, and radiating from the attachment of the basilar membrane. At this point there is generally a large blood-vessel; whilst between the spiral liga-

ment and the membrane of Reissner the periosteum contains pigment cells and a number of blood-vessels. *The floor of the ductus cochleæ* is formed of a narrow portion of the spiral lamina, and of the basilar membrane. This portion terminates in a border which is C-shaped when seen in section, the lower limb of the C being prolonged and tapering. This limb is the end of the osseous lamina; it is covered by a thin membrane. The upper portion of the C is the *limbus* of the spiral lamina, whilst the bay of the C is called the *spiral groove. The limbus* has a jagged edge, as it is raised into a number of tooth-like projections. *The organ of Corti* forms a portion of the epithelium covering the basilar membrane; it consists of an outer and inner set of stiff rod-like bodies. The feet of the rods rest upon the basilar membrane, whilst they incline towards each other until they meet by their heads. By the meeting of the rods an arch is formed over the basilar membrane; it is filled with endolymph. On the inner side of the inner rod, and the outer side of the outer rods, are epithelial cells with short hair-like prolongations, the *inner and outer hair cells;* the outer cells are more numerous and more elongated than the inner cells. The hair-like prolongations of the outer hair cells project through rings which surround the tops of the cells, and which are bounded by minute fiddle-shaped cuticular structures—*the phalanges. A reticular membrane* is thus formed, which covers this part of the organ of Corti. On either side of the two sets of hair cells the epithelium passes continuously into the simple layer of cubical cells, which is found in the spiral groove, and covering the outermost part of the basilar membrane. The whole organ of Corti is also covered by a thick and highly elastic *tectorial membrane. The inner rods* are smaller and more numerous than the outer rods; they may be compared to the upper portion of the human ulna; whilst *the outer rods* resemble the head and neck of a swan. The concavity of the inner rod receives the rounded portion of the outer rod, which would correspond to the back of a swan's head; whilst the beak of the swan becomes connected to the reticular lamina. Both rods are more slender towards their middle, and expand again, so as to rest by a widened foot upon the basilar membrane; both are longitudinally striated. In the head of the outer rod—and occasionally, also, in the inner rod—

is an oval nucleus, staining more deeply than the rest of the cell. (Schäfer.)

Structure of the wall of the membranous semicircular canals.—The wall consists, from without inwards, of (*a*) *an external fibrous layer*, containing numerous nuclei, blood-vessels, and irregular pigment cells. This layer is especially developed at the ends of the oval section where it coalesces with the ligamenta labyrinthi canaliculorum. (*b*) *The tunica propria*, which presents, after staining, a delicately striated granular appearance. (*c*) *Capilliform processes*, which project into the interior of the canal, except at the part where the membranous canal touches the bone. (*d*) *The epithelium*, a single layer of pavement epithelium cells investing the papillæ, and also continuous into the depressions between them.

In the ampullæ (*a*) *the fibrous layer* forms a loose meshed tissue, whilst (*b*) the *tunica propria* is so much thickened as to cause a rounded transverse projection into the cavity—*the crista acustica*, or *septum transversum*. (*c*) *The epithelium*, covering the crista acustica, consists (i) of long cylindrical cells, each with a large nucleus; these cells support the other nervous and epithelial elements, and rest upon the tunica propria; (ii) fusiform cells which lie between the columnar cells: each cell has a long stiff cilium, *the auditory hair*, and is in direct connection with the ultimate fibrillæ of the auditory nerve. (*d*) *The nerves*, after passing through the tunica propria, form a very delicate plexus in the epithelial layer.

THE NOSE.

Preparation.—Small pieces of the upper turbinal bones from the head of a freshly killed sheep, dog, or rabbit, should be hardened in $\frac{1}{6}$% chromic acid solution for a week, and in $\frac{1}{4}$% bichromate of potash, or in 1% osmic acid, for forty-eight hours. Sections may be made through the nasal region of a newt or young guinea-pig's head which has been previously hardened in chromic acid and spirit. The fresh tissue may also be treated with chloride of gold.

Structure.—In a vertical section through the septum nasi, the osseous portion is seen to be invested by *periosteum*, which is immediately covered by a thick layer of numerous and elongated *tubular glands*, some simple, others more complex,—the glands of Bowman. These glands contain an epithelium of granular spherical cells at the base; of a more polygonal and less granular form near the excretory duct. The ducts open on the surface between the elements of the external layer. The glands become less numerous and ultimately disappear at the point where the olfactory region passes into the ordinary mucous membrane, being replaced by the *mucous glands*. The glands are separated by ordinary *connective tissue*, in the deeper layers of which are *pigment cells* and free *pigment masses*, as well as *capillaries* and ramifications of the olfactory nerves. *The epithelium* is superficial, and consists of an external finely striated portion and an internal granular layer. In the newt the epithelial cells are separated into groups, by teasing after maceration for forty-eight hours in Müller's fluid. Each group consists of two kinds of cells; one kind, which is larger than the other, presents an elongated oval form, and is situated externally. *The olfactory cells*, the smaller of the two kinds, possess a large round nucleus and two very long fine processes, of which the thicker runs outwards, whilst the finer is directed inwards. The external process is composed of two substances, an outer which swells up under the influence of certain reagents, and an internal thread which remains unaffected. In man and mammalia generally the olfactory cells have no cilia. *The larger cells* are provided with oval nuclei, and extend through the whole thickness of the epithelial layer. The external portion of these cells is more or less cylindrical, and is striated longitudinally. A row of dots can be distinguished upon the external extremities. The trunks of the *olfactory nerve* run in the glandular layer either obliquely or horizontally. The ultimate fibrils of the nerve pass into the epithelial layer and probably into the olfactory cells.

THE EYE.

THE EYELIDS.

General Characters.—*The skin* of the eyelids consists of a thin corium; the papillæ are small, and the subcutaneous tissue very loose, containing numerous wide lymphatics; a few groups of fat cells are present. The sweat glands are small, and the hairs fine, with small sebaceous glands. At the anterior edge of the free margin of the lids the papillæ become larger, and the hairs are converted into eyelashes. Immediately behind the cilia are the ducts of *the glands of Moll*, which frequently open into the ducts of the sebaceous glands. The glands of Moll closely resemble in structure the sweat glands.

Next to the subcutaneous tissue are bundles of striated fibres of *the orbicularis muscle*, separated from each other by loose connective tissue, which occasionally contains fat cells. *The tarsal plate* is a dense felted mass of fibrous tissue which does not contain any cartilage: its anterior and posterior surfaces are intimately connected by bundles of connective tissue with the skin of the eyelid and with the conjunctiva.

Near the posterior edge of the free margin are the mouths of *the Meibomian glands*, arranged in a single row. The ducts are imbedded in the tarsal plate, and are in direct connection with saccular single or branched alveoli; the glands resemble sebaceous glands. At the posterior edge of the lid the stratum Malpighii becomes modified, and passes into the conjunctiva. A comparatively thick layer of muscle, the *musculus ciliaris Riolani*, intervenes between the mouths of the Meibomian glands and the eyelashes. *The Mucosa of the conjunctiva palpebræ* is a thick connective-tissue membrane, which generally contains a variable amount of diffuse adenoid tissue.

The conjunctiva palpebræ itself consists of one or two layers of small polyhedral cells, upon which is superposed a layer of longer or shorter conical or columnar cells, amongst which are frequently seen some goblet cells. Small *mucous glands* are embedded in the tarsal plate in the neighbourhood of the conjunctiva palpebræ. (Klein.)

The lachrymal gland is a serous gland; it is divided into

lobes and lobules by the connective-tissue capsule. The larger *interlobular ducts* are lined with a layer of thin columnar epithelium cells. *The intralobular ducts* are also lined with columnar cells, whose external portion is distinctly fibrillated, whilst the inner portion is only slightly striated : the nucleus is situated about the centre of each cell. The intermediate portions of the intralobular ducts, and the parts immediately opening into the alveoli, consist of fine tubes lined with a layer of flattened cell plates, which are often imbricated. *The alveoli* are more or less tubular, and are provided with lateral and terminal tubular or saccular branches. *The membrana propria* consists of homogeneous flat branched cells, from which septa extend between the cells of the glandular epithelium. *The glandular epithelial cells* form a single layer of polyhedral, cubical, granular-looking cells, each provided with a spherical nucleus. The distribution of *blood-vessels* is identical with that of the salivary glands. (Klein.)

CORNEA.

Preparation.—The anterior part of a human eye which has been hardened in 2% solution of bichromate of potash for a fortnight, and then in spirit, should be used.

To demonstrate the connective-tissue cells and nerves, the fresh cornea of a frog or rabbit should be cut out, and placed for about an hour and a half in chloride of gold solution ½%, then in slightly acidulated water, and exposed to the light for twenty-four hours or more, according to its intensity, and mounted whole (or in section in the case of the rabbit's cornea) in glycerin.

In order to stain the cell spaces, the fresh or living cornea of a pithed frog should be pencilled with solid nitrate of silver, stained and mounted in glycerin.

Structure.—Consists of five layers : (1) The superficial or conjunctival layer is composed of three or four distinct strata of nucleated cells of stratified *epithelium*, of which the lowest is columnar. (2) This layer merges into a thin homogeneous layer, the *anterior elastic lamina* (only a distinct layer in the human eye), which does not seem to differ from the substance of the cornea proper, except in its greater density and the absence of corneal corpuscles. (3) The *proper substance* of the cornea,

which is made up of alternating layers of fibrous tissue parallel to the surface. These are separated from one another by ground substance, in which are the cell spaces, of irregular branched form, which freely communicate with the cell spaces of the same, as well as of other layers. In the chloride of gold cornea, these cells appear as large and branched granular dark-red or black cells, with large oblong nuclei containing nucleoli. In the spaces, but not filling them up entirely, are the corneal corpuscles, branched cells of various forms. (4) Membrane of Descemet, or *posterior elastic lamina*,—a firm, structureless, but brittle, transparent membrane, covered by (5) a layer of endothelial or *epithelial cells*. There are no blood-vessels in a healthy cornea, except at the periphery. *Nerves* enter the proper substance of the cornea, and becoming transparent form a plexus, from which finer branches going forward form another "sub-epithelial plexus," from which, again, finer fibrils pass among epithelial cells, forming the "intra-epithelial plexus."

RETINA.

Preparation.—The posterior part of the eye of a pig (if no fresh human eye can be had) is hardened in Müller's fluid for a week, and then transferred to alcohol; pieces of the retina may then be stained in alcoholic logwood, and cut. Double staining with eosin and anilin green, or with anilin rose and anilin green helps to differentiate the layers. Another method is to place in 2% solution of osmic acid for four hours, then in water for one hour, to get rid of the excess of osmic acid, and finally in alcoholic logwood. The retina, thus treated, should be embedded in cacao-butter, instead of the ordinary wax.

Structure.—Consists of eight layers in the following order, from within outwards:—

1. *Layer of nerve fibres*, which is wanting at the yellow spot: the fibres consist of axis cylinders only; it diminishes in thickness anteriorly.

2. *Layer of nerve cells* (ganglionic layer), consisting of cells of a spheroidal or pyriform figure; one process of each extends into the first layer, and is doubtless continuous with it. From the other end of the cell, one or more processes extend outward for a variable distance into the next layer. In the yellow spot there are several layers of cells; elsewhere, only one layer.

3. *Inner molecular layer*, a dense network of various fibrils which appear as a thick stratum of granular-looking substance.

4. *Inner nuclear layer* consists of transparent nucleus-like bodies, of at least four kinds—(*a*) a few connected with the fibres of Müller (to be described below); (*b*) the largest number, like bipolar cells, one pole unbranched, passing inwards, and being connected with a nerve fibre,—the other, thicker and branched, running outwards, is supposed to break up into a plexus in the outer molecular layer; (*c*) unbranched cells found as a complete stratum at the innermost part; (*d*) scattered in the outermost part, are rounded, of large size, and have only one process.

5. *Outer molecular layer*, thinner than (3), but otherwise the same.

6. *Outer nuclear layer*, consists roughly of two kinds of corpuscles embedded in a reticular matrix: (*a*) connected with the rods are most numerous, and may be considered as dilatations midway in the centre of the fine rod fibres, have an elliptical striated nucleus, but no nucleolus; (*b*) connected with the cones are fewer, pear-shaped, not striated, and nearer the outer part of the layer in the thicker cone-fibre.

7. *Layer of rods and cones*, is composed of elliptical elongated bodies, *the rods*, and shorter, thicker, club-like bodies, *the cones;* each consists of two parts, inner and outer, of which the outer is transversely striated and smaller, and in the cones tapers to a point, whilst the inner is fibrillated externally, but homogeneous internally.

8. *Pigmentary layer*, or *uvea*, consists of a single stratum of hexagonal pigment cells.

Fibres of Müller consist of bands, which pass through all the layers of the retina, binding them together; they commence by a broad base, forming by their union the *membrana limitans interna;* and, at the outside of the retina, the *membrana limitans externa.* In the inner nuclear layer they give off processes which contain a clear, oval, and elliptical nucleus. In the outer nuclear layer they break up into fibrils, and partially enclose the rod and cone fibres.

Blood-vessels.—The arterial and venous branches are situated internally under the layer of nerve fibres. The capillaries are arranged in plexuses, with large meshes. They occur in the

inner molecular layer, one plexus being situated near the inner nuclear layer, the other near the layer of nerve cells. A plexus also exists in the inner nuclear layer, whilst another lies more superficially in the outer molecular layer. (Klein.)

CRYSTALLINE LENS.

Preparation.—(*a*) Harden the eye of a frog, from which the cornea has been removed, in a solution consisting of one part of fuming nitric acid, three parts of water, and one part of glycerin. At the end of twenty-four hours remove it from this solution, and allow it to remain for a day in water. Tease a portion of the lens thus prepared in glycerin, and mount it in Farrant's solution or glycerin. (Freud, Stirling.)

(*b*) Sections of the lens should be made from eyes which have been hardened for a fortnight in Müller's fluid, and afterwards in weak spirit.

(*c*) The capsular epithelium is best demonstrated by staining the uninjured lens of the frog in nitrate of silver, and afterwards mounting portions of the anterior capsule in glycerin.

Structure.—(*a*) *Of the capsule.* The portion which covers the anterior surface of the lens consists of a thick elastic layer, immediately behind which is a single layer of granular hexagonal epithelium cells, each of which is provided with an oval nucleus. The elastic lamina covering the back of the lens has no such lining epithelium, but is in close contact with the lens fibres. (*b*) *The lens* itself is composed of (1) *The lens fibres*, which are elongated bands running from the posterior to the anterior surface; they are broader behind than in front. Each fibre contains a nucleus, which is more distinct in the peripheral than in the central fibres. Every fibre is hexagonal when seen in transverse section, and is serrated along its narrow edge, the teeth of one fibre fitting into the notches of its neighbour. (2) *The interstitial substance* is like that of connective tissue, it is permeated by lymph channels.

IRIS.

Preparation.—The iris from an eye hardened in chromic acid and spirit mixture, together with the ciliary processes, can be cut by means of the freezing microtome; the operation

requires much care. The whole iris of a small animal may be mounted and examined in a recent state in saline solution.

Structure.—The iris is principally made up of connective tissue and blood-vessels. This forms the middle layer. In front it is covered by *endothelium*, which may contain pigment, a homogeneous basement membrane intervening. Behind there are similar layers, *i.e.*, an endothelial pigmentary layer, *the uvea*, and an intervening basement membrane. Around the inner border is a circular muscle of unstriped fibres, the *sphincter pupillæ*. Under the uvea, *i.e.*, between that and the iris proper, is a thin radiating membrane of muscle cells, which passes outwards from the sphincter. This is the *dilator pupillæ*. The *blood-vessels* of the iris are arranged in dense capillary plexuses in the tissue proper and on the sphincter. *The Nerves* form a plexus near the outer edge of the iris, from which pass off medullated fibres, which terminate in the dilator and in the anterior surface of the iris proper, and also non-medullated nerves to the sphincter.

CILIARY PROCESSES.

Structure.—The ciliary processes resemble the iris in structure, having a similar connective-tissue basis, containing branched pigment cells, covered by a transparent membrane, the *lamina vitrea*, whilst external to this is the uvea, differing in no way from the uvea of the retina. *The uvea* is covered by a single layer of columnar transparent cells. *The ciliary muscle* is connected with the outer part of the ciliary processes, and is made up principally of fibres radiating outwards, but partly of circular bundles.

CHOROID.

Structure.—Consists of the following coats:—

(*a*) *Lamina fusca*, a loose connective tissue, with corpuscles, both with and without pigment, branching or unbranched; and the lamina suprachoroidea, which is a continuation of the lamina fusca.

(*b*) *Stratum vasculosum*, in which are the large blood-vessels embedded in loose connective tissue.

(*c*) *An elastic layer* containing small arteries and veins, covered on each side by endothelium.

(*d*) *Membrana chorio-capillaris*, which contains the dense capillary meshwork, covered by cells, spindle-shaped and flattened, with or without pigment.

(*e*) *Lamina vitrea* (as above).

(*f*) *Uvea*, or retinal pigment.

SCLEROTIC.

Structure.—This coat is made up of dense fibrous tissue, the bundles of which in part cross and interlace. Between the bundles are connective-tissue corpuscles, almost precisely similar to those of the cornea. These cells are contained in spaces which form an anastomosing lymph-canalicular system. Non-medullated nerve fibres are said to exist in a dense plexus in the tissue. Near the ligamentum pectinatum iridis, at the corneo-scleral junction, is a circular canal, lined with endothelium ; it communicates indirectly with the lymph spaces mentioned above, and is called the canal of Schlemm.

CHANGES OBSERVED IN THE CELLS OF SECRETING GLANDS DURING DIGESTION AND REST.

The following facts* appear to be so important, that we have added it to this part of the book.

SALIVARY GLANDS.

(A) **Mucous glands**; *e.g., submaxillary of dog.* (*a*) *Before digestion commences.* Cells of alveoli do not stain readily with carmine. Protoplasm has become converted into a mucin-bearing substance, *mucigen.* Only a small amount of easily staining protoplasm remains around nucleus.

(*b*) *After prolonged stimulation of chorda tympani.*—Cells of alveoli stain better from a diminution of the mucigen and an increase of the protoplasm—are smaller, and sometimes no mucigen cells can be seen: cells stain deeply, are small, and are all composed of protoplasm alike.

(B) **Serous glands**; *e.g., parotid of rabbit, submaxillary of rabbit*—effects of stimulation. (*a*) *Of cerebro-spinal nerve* (*i.e.*, the auriculo-temporal in the one case, or chorda tympani in the other). Very slight changes in the microscopic appearance of the cells, even though in former case there is a copious secretion of watery saliva.

(*b*) *Of cervical sympathetic.*—There may be no extra secretion at all, but marked changes occur in the cells. During rest they are pale, transparent, almost without granules, stain badly, and have nuclei with irregular outlines, as if shrunken. After stimulation of the sympathetic the cells become turbid and very granular, stain readily, nuclei are large and round, and possess conspicuous nucleoli; the cells themselves are smaller.

STOMACH.

BOTH IN PEPTIC AND PYLORIC GLANDS.

(*a*) *Before a meal,* central cells are pale and finely granular, and do not stain well in carmine and other dyes.

* Epitomised from Foster's "Physiology." (3rd ed.)

(*b*) *During early digestion*, cells are swollen, turbid, and coarsely granular; stain better.

(*c*) *During a later stage of digestion*, cells are smaller and shrunken, more turbid and granular than before, and stain still better.

Peptic or parietal cells are swollen and enlarged, but otherwise unchanged during digestion.

PANCREAS.

(1) Each cell of the pancreas of a dog which has been fasting for thirty hours consists of two zones—*inner*, next the lumen of alveolus, which is finely granular, and not easily stained, and an *outer* (smaller), homogeneous or finely striated, easily stained with carmine. The nucleus lies partly in one zone, partly in the other, and is irregular in shape.

Six hours after food, *i.e.*, during full digestion, inner zone smaller, cells generally smaller, the outer zone larger, the whole cell stains better, and nucleus is large and spherical. (Heidenhain.)

In the living rabbit (Kühne and Lea):—

Quiescent pancreas—the cells are filled with granules, the outer transparent zone being very small, cells indistinct, margins of alveoli smooth, lumen obscure, blood supply scanty.

During digestion, margins of alveoli become indented, from bulging of cells, outline of cells distinct; granules pass into inner zone, and diminish, the whole cell becomes transparent from outer border inwards; blood supply increased.

Differences between phenomena presented by pancreas and stomach.—In pancreas, granular part does *not* stain so well as in stomach, the homogeneous does. Also, in pancreatic secretion, granules appear to be used up. If this is the case in gastric secretion, the granules are formed more rapidly than they are used up, and cease to be formed as digestion is completed.

PART II.

PHYSIOLOGICAL CHEMISTRY.

Apparatus and Reagents required.

Test tubes
Test-tube stand.
Retort stand.
Platinum foil and wire.
Three Berlin dishes.
Three beakers.
Sand bath.
Filter papers and funnels.
Sulphuric (H_2So_4), Nitric (HNO_3), Hydrochloric (HCl), Acetic ($C_2H_4O_2$), Tannic ($C_{14}H_{10}O_9$) Carbolic (C_6H_6O), and Boracic acids (H_3BO_3).
Magnesium chloride and sulphate ($MgCl_2$. $MgSO_4$).
Copper sulphate, 2%, 5% solutions ($CuSO_4$).
Sodium carbonate (Na_2CO_3).
Caustic potash, or soda (KHO or NaHO).
Ammonia (NH_4HO).
Millon's reagent. (Mixed mercury nitrate and nitrite?)
Potassium ferrocyanide, (K_4FeCy_6).
Potassic and mercuric iodide (KI,HgI_2).
Mercuric nitrate ($Hg(NO_3)_2$, and chloride ($HgCl_2$), mercurous nitrate $Hg_2(NO_3)_2$.
Lead acetate ($Pb(C_2H_3O_2)_2PbO$).
Calcium chloride ($CaCl_2$).
Alcohol.
Ether.

PART II.

PHYSIOLOGICAL CHEMISTRY.

THIS division of the book treats of the chief substances found in the animal body. The methods of preparing them, and of their tests, also of foods, and the action of the digestive juices upon them, of the secretions and excretions, and of calculi. For the sake of convenience the substances are treated of in the following order:—

(1) Proteids, or albuminous substances, which occur in the animal tissues and in food. The blood.
(2) Nitrogenous bodies, other than proteids.
(3) Carbo-hydrates.
(4) Oil and fats.
(5) Healthy urine, and its constituents.
(6) Abnormal urine.
(7) Calculi.

Proteids or **albumins** are bodies which are found to be present in all protoplasm, of which indeed they are the principal part. They contain the elements carbon, hydrogen, nitrogen (sulphur), and oxygen, in certain amounts, varying within the limits annexed in the table below, and present certain general properties. Protoplasm may also contain carbo-hydrates, fats, nitrogenous bodies other than proteids, as gelatin, chondrin, etc., and nitrogenous and other derivatives, such as urea, but proteids "are indispensable constituents of every living, active, animal tissue, and indissolubly connected with every manifestation of animal activity."*

* Gamgee. Phys. Chemistry, vol. i. p. 1.

PROTEIDS or ALBUMINS are thus classified :—

I. **Native Albumins.** Egg Albumin. Serum Albumin.

II. **Derived Albumins.**—Acid Albumin. Alkali Albumin. Casein.

III. **Globulins.**—Globulin. Fibrinoplastic Globulin. Fibrinogen. Myosin. Vitellin.

IV. **Fibrin.**

V. **Peptones.**

VI. **Coagulated Proteids.**

VII. **Lardacein** or **Amyloid** substance.

Composition of Proteids :—

C	H	O	N	S
52·7	6·9	20·9	15·4	0·8
to	to	to	to	to
54·5	7·3	23·5	16·5	2·0

Exist generally in a soluble and insoluble form.

General Reactions :—

(i) *Turn yellow on heating with strong nitric acid; colour deepens on addition of ammonia* (xanthoproteic reaction).

(ii) *With Millon's reagent (nitrate and nitrite of mercury), a pink precipitate or mere coloration, either directly or on boiling.*

(iii) *With a drop or two of a weak solution of copper sulphate and caustic soda, a violet colour.*

(iv) *With acetic acid and ferrocyanide of potassium, a white precipitate.*

(v) *Add acetic acid in excess, then sodium sulphate; boil, and a white precipitate will be formed.*

All act on polarised light.

Method of preparing Millon's Reagent.—Take equal parts by weight of pure mercury and of nitric acid, add the acid to the mercury, place in a ventilated cupboard, and leave until the mercury is dissolved—if necessary, warming slightly. Then add twice its bulk of water. After a time a crystalline white precipitate falls, and the supernatant fluid is decanted.

SOLUBILITIES OF PROTEIDS.

a. SOLUBLE IN WATER.

Native Albumins.

Peptones.

Not coagulated on heating.
Peptones.

Coagulated on heating.
Native albumins.

b. INSOLUBLE IN WATER.

Globulins.
Derived albumins.
Fibrin.
Coagulated proteids.
Lardacein.

Of *b.*

c. Soluble in dilute saline solutions.
Globulins.

d. Insoluble.
Derived albumins.
Fibrin.
Coagulated proteids.
Lardacein.

Of *d.*

e. Soluble in dilute acids or alkalies.
Derived albumins.

f. Insoluble.
Fibrin.
Coagulated proteids.
Lardacein.

Of *f.*

g. Soluble on boiling with dilute hydrochloric acid.
Fibrin.

h. Insoluble.
Coagulated proteids.
Lardacein.

Of *h.*

Soluble by action of gastric or pancreatic juice.
Coagulated proteids.

Insoluble.
Lardacein.

CLASS I.—NATIVE ALBUMINS.

Egg Albumin.

Preparation.—To white of egg in a test tube add about three or four times its volume of water, shake up and filter. Use the solution for following experiments :—

Experiments.—Evaporate to dryness at 40° C

(Dry Albumin ; a yellow, transparent, glassy mass, soluble in water.)

Coagulation on simply boiling a strong solution.

Precipitate on addition of strong acids, nitric, hydrochloric, or sulphuric acids (HNO_3, HCl,H_2SO_4).

No precipitate with organic acids except tannic or carbolic.

Precipitation by mercuric chloride, lead acetate, copper sulphate and silver nitrate without coagulation, the precipitate soluble in slight excess.

Coagulation by ether.

Coagulated albumin is dissolved by caustic alkali.

Tests for Albumin.—Albumin precipitated from dilute solution by adding acetic acid and ferrocyanide of potassium (a most delicate test).

Heller's test.—Pour a little strong nitric acid into a test tube, and add gradually dilute solution of albumin : albumin precipitated at point of contact with the acid, in the form of a fine ring.

Serum Albumin.

Preparation.—Dilute blood serum with water, add ether, shake up, filter, and test filtrate with strong nitric acid.

Differs from egg albumin in not being coagulated by ether.

Also differs from egg albumin in being easily precipitated by hydrochloric acid, and in the precipitate being easily soluble in excess of this acid.

Serum albumin, either in the coagulated or precipitated form, more soluble in excess of strong acid than egg albumin.

All albumin is very slightly diffusible.

Experiment.—Solution of albumin should be placed on dialyser, and the outside liquid tested for albumin.

Salt should be mixed with albumin solution, and tested for in the outside liquid.

CLASS II.—DERIVED ALBUMINS

Are insoluble in water and in solutions of chloride of sodium ($NaCl$), but are soluble in dilute acids and alkalies.

Acid Albumin.—If a small amount of dilute hydrochloric acid (HCl 0·4 to 1%) or acetic acid be added to either egg or

serum albumin, there is no precipitate or coagulation on heating. This is due to the conversion of the native into an acid albumin.

On *exactly* neutralising the solution, a flocculent precipitate is produced. This is to be accomplished by adding to the acid albumin solution a little aqueous solution of litmus, and then dropping a weak solution of caustic potash from a burette, until the red colour becomes almost bleached.

The albumin has become insoluble in water and neutral saline solutions.

It is soluble in acids and alkalies. Insoluble in chloride of sodium (NaCl) solutions.

Acid albumin is also made by dissolving coagulated native albumin in strong acid, or by dissolving any of the globulins in acids.

Partial coagulation of acid albumin solution on boiling in lime-water, and further precipitation on addition to boiled solution of calcium chloride ($CaCl_2$), magnesium sulphate ($MgSO_4$), or sodium chloride (NaCl.)

Alkali Albumin.—If solutions of albumin be treated with dilute fixed alkali, coagulation on heating is prevented. This is due to the conversion of the native into an alkali albumin.

Alkali albumin thrown down on neutralising solution, except in the presence of alkaline phosphates, in which case the solution must be distinctly acid before a precipitate falls.

To differentiate between Acid and Alkali Albumin, the following method is useful :—

(1) Alkali albumin is *not* precipitated on *exact* neutralisation, if sodium phosphate has been previously added.

(2) Acid albumin is precipitated on exact neutralisation, whether or not sodium phosphate has been previously added.

Solid alkali-albumin may be prepared by adding caustic soda or potash, drop by drop, to undiluted egg albumin, until the whole forms a jelly. This jelly is soluble in dilute alkalies on boiling.

Casein.—Obtained from milk by the following process :—

Dilute the milk with three to four times its volume of water, add sufficient dilute acetic acid to render the solution distinctly acid, but not more than a few drops, and filter off the casein. To purify it, wash with alcohol and then with ether.

Casein may also be prepared by adding to milk an excess of

crystallized sulphate of magnesium, which causes it to separate out.

Casein is similar to alkali albumin, but differs in (1) yielding sulphide when heated with KHO to 110°; (2) yielding a phosphorus-containing body when digested with gastric juice.

Casein is slightly soluble in dilute caustic alkalies and acids; more so in hydrochloric than in acetic acid.

It is retained to a considerable extent in solution, by the pre sence of alkaline phosphates.

MILK.

Examination of Milk.—Examine a drop of milk under the microscope with a high power. See that it consists of fat globules of different sizes in a clear fluid. Add dilute acetic acid by irrigation, and observe the coalescence of the globules owing to the excess of acid dissolving their casein membrane.

Test the alkaline reaction of *fresh* milk; it becomes acid on standing, owing to the formation of lactic acid, whilst in consequence the casein separates.

The constituents whose presence is to be demonstrated are *Oil* or *Fat*, *Casein*, *Serum Albumin*, *Lactose* or *Milk Sugar* and *Salts*.

Fat.—To a portion of milk, add its own volume of caustic potash or soda, and warm the solution *gently*, the oil globules will be set free from their enveloping casein. Add ether, shake the mixture, and allow it to stand. The fat will be dissolved in the ether, and will form with it a clear superstratum. Remove the transparent top layer with a pipette, evaporate off the ether, the oil will be left, and will give the *characteristic greasy spot when dropped upon paper.*

Casein.—Dilute some of the milk with its own bulk of water; add a few drops of dilute acetic acid until a slight granular precipitate is formed. Warm the solution *gently* to 40° C., and a copious flocculent precipitate will appear. Filter off, and lable the precipitate A.

Albumin.—Boil the clear filtrate (from A), a coagulum of albumin will be formed. Filter this off, and label the precipitate B.

Exactly neutralise the clear filtrate (from B), with caustic

potash or soda, a precipitate of albumin which is soluble in acids will appear.* Filter off precipitate, and label it C.

Lactose.—Test the clear filtrate (from C) by Trommer's test (page 134) for sugar.

To precipitate A in a test tube add nitric acid, the precipitate is dissolved; boil, and when cold add strong ammonia, the solution becomes orange-coloured (*xanthoproteic test*).

To a second portion of precipitate A add sodium phosphate, the precipitate will be dissolved; add dilute acetic acid to the solution till a neutral reaction is just obtained, no precipitate occurs; add more acetic acid, a precipitate is thrown down (since acid albumin is now present), *casein or alkali albumin is present.*

To a third portion dissolved in caustic soda or potash add lead acetate, a black precipitate of lead sulphide is formed. Therefore sulphur is present; consequently *casein*, and not artificially produced alkali albumin, is present.

Test the precipitate on filters B and C, by boiling with *Millon's reagent;* a pink coloration will in each case be produced, showing that albumin is present.

Test a second portion of the clear filtrate from C for (1) *phosphates*, by the addition of ammonio-sulphate of magnesia, a precipitate is formed; and for (2) *chlorides* by the addition of silver nitrate to another portion which has been acidulated with nitric acid, a curdy precipitate will fall.

CLASS III.†—GLOBULINS

Are insoluble in water: soluble in very dilute acids and alkalies, as well as in 1% solution of sodium chloride, and in solutions of other neutral salts.

They differ from native albumins in not being soluble in distilled water, and from derived albumins in being soluble in neutral saline solutions.

Are converted by acids and alkalies into acid and alkali albumin respectively.

† This acid albumin has been produced by the previous acidification of the milk in the precipitation of casein.

* The properties and preparations of bodies of this class need not be practically demonstrated by the junior student.

(1) **Globulin or Crystallin.**—Obtained from the crystalline lens.

(2) **Myosin.**—May be prepared from dead muscle by removing all fat, tendon, etc., and washing repeatedly in water, until the washing contains no trace of proteids, and then treating with 10% solution of sodium chloride, which will dissolve a large portion into a viscid fluid, which filters with difficulty. If the viscid filtrate be dropped little by little into a large quantity of distilled water, a white flocculent precipitate of myosin will occur.

(3) **Fibrino-plastic Globulin, or Paraglobulin.**—Blood serum diluted with ten vols. of water, and carbonic acid gas passed rapidly through, fine precipitate collected on filter, and washed with water containing carbonic acid gas.

Also prepared by saturating serum with sodium chloride or magnesium sulphate.

Is very soluble in dilute saline solutions, from which it is precipitated by carbonic acid gas and dilute acids.

Its solution is coagulated at 70° C.

Even dilute acids and alkalies convert it into acid or alkali albumin.

Can be used to form fibrin.

(4) **Fibrinogen.**—Its general reactions are similar to those of paraglobulin.

Preparation by similar processes from fresh hydrocele fluid as those mentioned for preparing paraglobulin from serum.

Its characteristic property is that, when mixed with paraglobulin, it forms fibrin.

(5) **Vitellin.**—Obtained from yolk of egg.

(6) **Globin.**—The proteid residue of hæmoglobin.

CLASS IV.—FIBRIN.

A soft, white, fibrous, and very elastic substance, obtained from blood-clot by washing with large amount of water.

Differs from all other proteids, in having a filamentous structure. Examine with microscope.

Has similar chemical properties to coagulated albumin.

Is insoluble in water and dilute saline solutions, slightly soluble in concentrated saline solutions, soluble in strong acids and alkalies on boiling.

Fibrin formed by combining (i) fibrino-plastic globulin and fibrinogen; (ii) globulin from blood serum with hydrocele fluid.

CLASS V.—PEPTONES

Are obtained by the action of gastric juice, or of pancreatic juice on albumins, gelatin, etc.

Properties.—Are very soluble in water.

Are not precipitated on adding acids or alkalies.

Are not precipitated on boiling.

Are insoluble in alcohol and ether, but are only precipitated with difficulty by alcohol.

Are precipitated by mercuric chloride, the precipitate being soluble in excess of the reagent, by lead acetate, and by tannin, the latter precipitate being soluble in dilute acid.

With strong solution of caustic soda and a trace of copper sulphate they give a red colour; with excess of the salt, or with a drop of dilute Fehling's fluid (p. 143), a violet colour.

Are very diffusible; experiment with dialysing apparatus.

Gastric juice.—Chief constituents are a ferment (pepsin), and a free acid (hydrochloric).

Can be obtained by means of a gastric fistula. (Brunton, Hdbk., pp. 477, 478.)

Glycerin extract of gastric mucous membrane is made by taking the washed mucous membrane of a pig or some other animal, cutting in pieces, and placing in concentrated glycerin. The glycerin has the property of dissolving the pepsin.

Digestive action of Pepsin.—To some fibrin or albumin in a test tube add a trace of pepsin and a little ·2% hydrochloric acid, and warm gently; in a few minutes filter, and test the filtrate for peptones.

Dr. Grützner adopts the following method for estimating the digestive power of pepsin:—Fibrin is soaked in a solution of carmine, and is preserved in glycerin. When required for experiment, it is placed in a 0·2% solution of hydrochloric acid after it has been freed by washing from the glycerin, until a jelly-like red mass results. The jelly is readily soluble in the digestive juices, which it colours red as it dissolves. The greater the digestive power of the ferment, the more rapidly, *cæteris paribus*, does the fibrin dissolve, and the sooner conse-

quently does the solution become stained. The digested solution is compared with a standard colour solution made by mixing ammonia and carmine, 1 cc. of each, with 100 cc. of glycerin. The test is a delicate one.

CHEMISTRY OF THE BLOOD.

Test *the alkaline reaction of the blood* in the following way, as recommended by Prof. Schäfer :—A drop of blood, obtained by pricking the finger, is placed upon the smooth coloured surface of a piece of dry, faintly reddened, glazed litmus paper,* and after a few seconds is wiped off with the corner of a handkerchief or clean linen rag moistened with water. The place where the blood has stood is seen to be marked out as a well-defined blue patch upon the red or violet ground.

The coagulation of blood is retarded by freezing and by the presence of neutral salts.

Experiments.—Draw a few drops of blood from the finger into a watch-glass previously cooled in a freezing mixture ; no coagulation takes place at the freezing temperature. Add a drop of blood to a little saturated solution of sulphate of soda in a watch-glass ; the blood does not coagulate.

Two cc. of blood are placed in a platinum capsule, which is surrounded by alternate layers of pounded ice and salt. The capsule is allowed to remain until the blood is frozen, it is then removed, and the solid mass of frozen blood is gradually thawed. The blood on again becoming liquid will be found to be darker in colour and more transparent than it was previous to congelation. It is in the *lake* condition, owing to the discharge of the hæmoglobin from the corpuscles into the plasma.

Blood Spectrum.—Examine the blood with *spectroscope.* Observe the characteristic spectrum of oxy-hæmoglobin.

Try the action of reducing agents † on blood, and notice the production of the spectrum of reduced hæmoglobin.

Try also the action of carbonic oxide (CO) on the blood, and

* To be obtained of Messrs. Townson & Mercer, Bishopsgate Street, London, E.C.

† A convenient form of reducing agent is Stokes' Fluid, which is a solution of ferrous sulphate to which ammonium hydrate (NH_4HO) has been added, after the previous addition of sufficient tartaric acid to prevent precipitation.

observe the alteration of the spectrum, and that reducing agents produce no change in it. The effect of other gases may also be tried.

Crystals of oxy-hæmoglobin are readily obtained from the blood of the rat by placing a drop on a glass slide, adding a little distilled water or chloroform, and covering with a cover glass. The crystals are needle-shaped. Crystals of oxy-hæmoglobin may be obtained from the blood of guinea-pigs (see also p. 46), squirrels, cats, dogs, men, and rabbits, by freezing in the manner above described for producing the lake condition. In the pig and frog the crystals are only obtainable after the blood has been repeatedly frozen and thawed.

Blood serum is the plasma from which the fibrin has separated. It may be obtained in a very pure condition by means of the centrifugal machine.* More commonly, however, blood is allowed to coagulate, the clot is washed, and is suspended in a calico bag; the serum, as it exudes from the clot, passes through the calico, and is received into a vessel. The first drippings are rejected.

Test the serum for serum albumin (p. 124), and for paraglobulin (p. 128). Serum also contains neutral fats, lecithin, cholesterin, sugar, urea, uric acid, and other extractives, as well as certain inorganic salts.

Tests for blood.—Formation of hæmin crystals, p. 45.

The guaiacum test, p. 144.

The methods of counting the number of blood corpuscles, and of estimating the quantity of hæmoglobin present, are described in Part III.

(2) NITROGENOUS BODIES OTHER THAN PROTEIDS.

Gelatin is contained in bones, teeth, fibrous connective tissue, tendons, ligaments, etc.

Contains more nitrogen and less carbon than albumin.

It is an amorphous, transparent substance.

It is distinguished from the albumins by not being precipitated by potassium ferrocyanide (K_4FeCy_6), and by not being coagu-

* "Physiological Chemistry of the Animal Body." Gamgee. Vol. i., p. 58.

Is a soft white powder, consisting of rounded granules, having an organised structure.

Size of granules varies according to the plant from which they come.

Starch is insoluble in cold water, in alcohol, and in ether.

On boiling with a large amount of water for some time, it becomes soluble, and can be filtered.

Soluble and insoluble starch coloured blue by dilute solutions of iodine. On heating this compound with water, the colour disappears, but returns on cooling.

Starch is obtained from potatoes, by scraping and washing the scrapings, or from flour, by washing flour tied up in a bag.

Glycogen ($C_6H_{10}O_5$).

White amorphous powder resembling starch. Is soluble in cold water, turns brown with iodine.

Ferments or dilute acids convert it into grape sugar.

It occurs in the liver, and after death is found as grape sugar.

Liver should be boiled with a small amount of water, and the solution should be then filtered and tested for grape sugar.

For method of preparing glycogen, see p. 145.

Grape sugar ($C_6H_{12}O_6$).

Occurs widely diffused in the vegetable kingdom, and also in diabetic urine, in the blood, etc.

Is obtained by treating honey with cold alcohol, the grape sugar remaining insoluble.

Is easily soluble in water. Is not nearly so sweet as cane sugar.

Not easily charred by strong sulphuric acid.

Tests.—*Trommer's test.* Heated with copper sulphate and caustic soda, cuprous oxide is precipitated. In making the experiment, to the solution of grape sugar add first the caustic potash, then the copper sulphate, drop by drop, as long as the heat the solution gently (p. 143).

precipitate formed readily dissolves on shaking the tube, then

Moore's test.—A solution of grape sugar becomes brown on heating with caustic soda.

Fermentation test.—It is converted into carbonic acid and alcohol by yeast. $C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$ (p. 143).

Bismuth test.—Add a little bismuth oxide or subnitrate and an excess of caustic potash or soda to the suspected solution in a

test tube, and heat—if sugar be present, the bismuth oxide will be at first grey and then black.

Starch boiled for some time with water and sulphuric acid is converted into dextrin (British gum) and then into grape sugar; thin solution to be tested with iodine, and also with potash and copper sulphate.

Starch converted into sugar by saliva.

Quantitative estimation of grape sugar by Fehling's solution, or by saccharimeter. (Vide Ganot's Physics, 6 edit., p. 585.)

Saliva.—Can be obtained by filling the mouth with ether vapour. The saliva should be collected in a clear vessel, and filtered.

Characters.—Transparent or opalescent. Deposits a white precipitate on standing. More or less frothy and viscid.

Under the microscope is seen to contain epithelium (squamous, see p. 46) and round salivary cells.

Contains a ferment (ptyalin), together with albumin, mucin, and globulin; and inorganic salts, among which is sulpho-cyanide of potassium.

Test.—Add a drop of perchloride of iron (Fe_2Cl_6) to some concentrated saliva in a test tube; a red colour will appear, bleached by a solution of mercuric chloride ($HgCl_2$), but not by hydrochloric acid (HCl). This coloration is due to the sulpho-cyanide generally present.

Action of Saliva on Starch.—Add saliva to some fresh starch mucilage in a test tube, and warm gently for half an hour in a water bath (to 38° C), the starch will gradually be converted into sugar. Apply Trommer's test.

Shew that the action is delayed by cold, and by strong acids, and altogether stopped by strong alkalies.

Milk sugar (Lactose, $C_{12}H_{24}O_{12}$).

Important constituent in milk (p. 127). Is much less soluble in water than grape sugar. Only slightly sweet in taste. Reduces copper salts like grape sugar.

Inosit ($C_6H_{12}O_6$), a non-fermentible variety of glucose occurring in the heart and voluntary muscles, as well as in beans and other plants. It crystallizes in the form of large colourless monoclinic tables, which are soluble in water, but insoluble in alcohol or ether. Inosit may be detected by evaporating the

solution containing it nearly to dryness, and by then adding a small drop of a solution of mercuric nitrate, and afterwards evaporating carefully to dryness, a yellowish-white residue is obtained ; on further cautiously heating, the yellow changes to a deep rose colour, which disappears on cooling, but reappears on heating. If the inosit be almost pure, its solution may be evaporated nearly to dryness. After the addition of nitric acid, the residue mixed with a little ammonia and calcium chloride, and again evaporated, yields a rose-red coloration.

(4) OILS AND FATS.

Neutral substances. Composition, that of a compound ether. (Glycerin $C_3H_5(OH)_3$ being the alcohol.) They have a lower sp. gr. than water, from 0·91 to 0·94.

They give a greasy stain on paper. Are insoluble in water. Easily soluble in ether, chloroform, turpentine, and alcohol.

Glycerin ($C_3H_5(OH)_3$), a viscid liquid, soluble in water and alcohol, insoluble in ether.

Has a characteristic sweet taste.

Dry acid potassium sulphate heated with a little glycerin in a test tube yields *acrolein*, a body of unpleasant and pungent smell.

$$C_3H_8O_3 - 2H_2O = C_3H_4O.$$

Olive oil, glycerin oleate ($3(C_{18}H_{33}O_2)$, C_3H_5), heated in a test tube with water, dissolves on addition of NaHO; glycerin is liberated, and sodium oleate is formed. Both are soluble in water.

Olive oil, heated in a basin with water and lead oxide, and well stirred, glycerin is liberated, and lead oleate (lead plaster) formed.

Olein.		Soda.		Sodium Oleate.		Glycerin.
$3(C_{18}H_{33}O_2)$, C_3H_5	+	$3NaHO$	=	$3(C_{18}H_{33}O_2)$, Na_3	+	$C_3H_5O_3$, H_3

Fat (glycerin stearate) heated in a test tube with water, melts, and on addition of caustic alkali saponifies, a stearate of sodium and glycerin forming. Sodium chloride added to this solution, soap (sodium stearate) separates out.

Acid added to another portion, sodium stearate is decomposed. Stearic acid separates out.

To another portion, add a solution of calcium chloride; calcium stearate is precipitated.

BILE.

An alkaline fluid, sp. gr. 1,018 to 1,020. Of a colour varying from deep yellow to green or brown.

Bile contains mucin, bile pigments, bile acids in combination with sodium, cholesterin, lecithin, and inorganic salts, phosphates of sodium, calcium and iron, sodium chloride, and traces of copper.

Action of the Bile.—Bile shaken up with oil divides it into very small globules (an emulsion formed).

These can, under very slight pressure, pass through animal membranes; oil alone cannot.

Bile precipitates pepsin and peptone.

Experiments.—Add a considerable quantity of bile to a solution of fibrin digested in artificial gastric juice; a precipitate of pepsin and peptone will fall, and the solution will be found to have lost its proteolytic properties.

Take two filters, wet one with water, the other with bile, and pour a small and equal amount of oil on each. Oil passes slowly through the one wetted with bile, not at all through the other.

Bile acids, Glycocholic ($C_{26}H_{43}NO_6$), and Taurocholic ($C_{26}H_{45}NSO$).

Preparation.—Mix bile, which has been evaporated to one-fourth its bulk, with animal charcoal, evaporate to perfect dryness on a water bath, and extract it, whilst still warm, with absolute alcohol. The alcoholic filtrate should be colourless; if this is not the case, more charcoal must be added. The alcohol is distilled off, and the dry residue is treated with absolute alcohol. The alcohol is then filtered off, and to the filtrate anhydrous ether is added as long as a precipitate is thrown down. The solution and precipitate are to be set aside in a closely stoppered bottle for some days, when crystals of *bilin* will be produced. If the reagents were not perfectly anhydrous, a gelatinous mass will be formed, but no crystals.

Bilin consists of glycocholic and taurocholic acids, which may be separated by dissolving in water, and adding first solution of neutral lead acetate, and then a little basic lead acetate. This combines with the glycocholic acid, and forms an insoluble lead glycocholate. Filter, and add to the filtrate lead acetate and ammonia; a precipitate of lead taurocholate will be formed, which may be filtered off. In either case the lead may be got rid of by suspending or dissolving in hot alcohol, adding hydrogen sulphide, and filtering.

Cholesterin.—Contained in bile, gall-stones, nervous matter, etc.

Preparation.—Usually by extracting powdered gall-stones with ether or boiling alcohol. Cholesterin crystallizes out on evaporation of the extract. The insoluble residue consists of bile, colouring matter, and mucin.

Properties and Tests.—Crystallizes in rhombic plates. Examine under the microscope.

Insoluble in water and cold alcohol, freely soluble in boiling alcohol and in ether.

Gives a red colour with strong sulphuric acid and with nitric acid and ammonia.

Gives a greenish blue to violet with sulphuric acid and iodine or zinc chloride, and with sulphuric acid and chloroform a play of colours, beginning with blood-red, and ending with green, afterwards disappearing.

Tests for Bile.—*Pettenkofer's Test for Bile Salts.* Add a few grains of white sugar, or a drop or two of syrup, to a solution of bile in a test tube, shake well, add strong sulphuric acid, and cool. A reddish purple colour is produced.

Gmelin's Test for Bile Pigments.—Place a drop of bile on a white plate, and add a drop of strong yellow nitric acid to it. A play of colours is produced. Green, blue, red, violet, and yellow, appear in succession.

ACTION OF PANCREATIC JUICE ON FOOD-STUFFS.

1. *It converts starch into grape sugar.* Add some aqueous extract of pancreas to starch mucilage in test tube, warm gently, and test solution for grape sugar (p. 134).

2. *It emulsionizes fat.* Shake up some aqueous extract of pancreas with olive oil in a test tube, an emulsion is formed.

3. *It decomposes fats, liberating fatty acids.* If the emulsion be allowed to stand for some time, it becomes acid, owing to the liberation of the fatty acid.

4. *It converts proteids into peptones.* To boiled fibrin in test tube add some glycerin extract of pancreas, diluted with 1% solution of sodium carbonate, and expose to a temperature of 40° C. for an hour; then filter, neutralize filtrate with acetic acid, and test for peptones. The main difference between the action of pancreatic juice and the action of gastric juice on proteids is that part of the peptone formed by the former may be broken up into leucin and tyrosin.

5. *It curdles milk*, and very quickly dissolves the casein.

Preparation of Leucin and Tyrosin.—These bodies may be obtained by digesting fibrin for ten to twelve hours with pancreatic juice. The albumin is precipitated by slightly acidulating, boiling, and filtering the solution. The filtrate is then evaporated to a small bulk, and heated with strong alcohol to precipitate the peptones. On again filtering, an extract is obtained, from which leucin and tyrosin crystallize. The two bodies can be separated from each other by the addition of boiling alcohol, in which leucin is soluble, and from which it can be recrystallized. (*Sanderson.*)

(5) HEALTHY URINE, AND ITS CONSTITUENTS.

Urea (CON_2H_4).—The most characteristic constituent of urine.

Properties.—Soluble in alcohol and water. Crystallizes in transparent four-sided prismatic needles, terminated by one or two oblique facets.

Evaporate solution on a glass slide, and examine with microscope.

Precipitation of urea by mercuric nitrate, in absence of sodium chloride, but not in presence of excess of that salt; also by nitric acid.

To a strong solution add concentrated nitric acid (HNO_3), which is free from any trace of nitrous acid. Urea nitrate

($CON_2H_4HNO_3$) separates out in the form of six-sided tables. Examine with microscope.

Strong solution of oxalic acid added to urea solution, urea oxalate ($CON_2H_4\ C_2H_2O_4$) separates out in the form of tabular or prismatic bundles. Examine with microscope.

Uric acid ($C_5H_4N_4O_3$).

Insoluble in cold water, very slightly soluble in hot. Dissolves on addition of caustic alkalies, forming urates.

The lithium urates the most soluble.

Test.—Add a drop of strong nitric acid to uric acid or a urate; evaporate to dryness over water bath, or at a temperature not exceeding 40° C. *Alloxan* ($C_4H_2N_2O_4$) is formed, as a reddish coloured residue. Add a drop of ammonia solution, and the purple colour of *murexide* ($C_8H_8N_6O_6$) is produced.

Healthy urine is a perfectly transparent, amber-coloured liquid, with a peculiar, but not disagreeable odour, a bitterish taste, and slightly acid reaction. Sp. gr. varies from 1,015 to 1,025.

Urine Test for Urea.—Evaporate urine to half its bulk, and add strong HNO_3; impure urea nitrate separates out.

Quantitative estimation of Urea *by means of Russell and West's Test.**—It consists of (*a*) a water bath supported by three iron bands, arranged as a tripod. The bath is provided with a cylindrical depression, and with a hole, into which fits a perforated india-rubber cork; (*b*) a bulb tube with a constricted neck; (*c*) a glass rod provided with an india-rubber band at one extremity; (*d*) a pipette of five cubic centimeters' capacity; (*e*) a graduated glass collecting tube; (*f*) a spirit lamp; (*g*) a wash bottle with distilled water; (*h*) hypobromous solution. The hypobromous solution is to be made in the following way: three and a half ounces (100 grams) of solid caustic soda is dissolved in nine ounces (250 grams) of distilled water. When the solution is cold, seven drachms (25 cc.) of pure bromine is to be added carefully and gradually. The mixture is not to be filtered: it keeps badly, and for this reason it should be made shortly before it is required; or the solution of caustic soda in water may be made in large quantities,

* The apparatus required may be obtained of Cetti & Co., Brooke Street, Holborn, London, W.C.

as it does not undergo any change, the bromine in the proper proportion being added at the time it is required for use.

Fill the pipette to the mark on the stem with the urine to be examined; pour the five cubic centimeters of urine thus measured out into the bulb; fill up the bulb tube as far as the constricted neck with distilled water from the wash bottle; insert the glass rod (*c*) in such a way that the india-rubber band at the extremity fills up the constricted neck; the diluted urine should exactly occupy the bulb and neck of the tube, no bubble of air being below the elastic band on the one hand, whilst on the other the fluid should not rise above the band; in the former case a little more water should be added, in the latter a fresh portion of urine must be used, and the experiment repeated. After adjusting the glass rod, fill up the rest of the bulb tube with hypobromous solution; it will not mix with the urine so long as the rod is in place. The water bath having been previously erected, and the india-rubber cork fixed firmly into the aperture, the bulb tube is to be thrust from below through the perforation in the cork. The greater part of the tube is then beneath the water bath, the upper extremity alone being grasped by the cork. Fill the water bath half full of water, fill also the graduated glass tube (*e*) with water, and invert it in the bath; in doing this no air must enter the tube, which when inverted should be completely filled with water. Now slide the graduated tube towards the orifice of the bulb tube, at the same time withdrawing the glass rod which projects into the bath through the cork. At the instant that the rod is withdrawn the hypobromous solution mixes with the diluted urine and a decomposition takes place according to the following formula:—

$$CON_2H_4 + 3NaBrO + 2NaHO = 3NaBr + 3H_2O + Na_2CO_3 + N_2.$$

Urea + sodium hypobromite + caustic soda = sodium bromide + water + sodium bicarbonate + nitrogen.

The nitrogen thus produced is given off as gas, and displaces the water in the graduated tube, which is held over it. The gas is at first evolved briskly, but afterwards more slowly; to facilitate its evolution, the bulb of the tube may be *slightly warmed* with a spirit lamp; as a rule, however, this is unnecessary. After ten minutes, the amount of water displaced by the gas should be read off on the tube, which is divided into tenths. Each number on the tube represents one gram of urea in 100 cc.

of urine. Normal urine should yield roughly 1·50 parts of nitrogen by this test. If 5 cc. of urine gives off more nitrogen than fills the tube to III, dilute the urine with an equal volume of water, and take 5 cc.; read off and multiply by two. If the urine contain albumin, heat it with two or three drops of acetic acid, filter, and take 5 cc. of the filtrate.

(6) UNHEALTHY OR MORBID URINE.

Albumin present.—If the urine be neutral or acid, the albumin is precipitated on boiling.

If alkaline, render slightly acid with nitric acid or acetic acid, and boil.

Coagulated albumin dissolved by caustic alkalies, and re-precipitated by nitric acid.

Care must be taken in testing for albumin in urine, that the test tube which is employed be clean and free from acid. Perform the following experiment to show the necessity of this precaution. To a small quantity of albuminous urine add an excess of strong nitric acid; a precipitate of albumin is thrown down. Pour the contents of the test tube away, and *without* washing it, fill it up with a fresh sample of the albuminous urine. After allowing it to stand five minutes, boil the solution, when, although albumin is known to be present, no coagulum will be formed, since the acid remaining in the test tube from the previous experiment has been sufficient to convert the albumin into acid albumin. The experiment will frequently, however, be unsuccessful, unless a large excess of the strong acid has been first added.

A more delicate test.—Acidify urine with acetic acid; mucus will be precipitated if present. Filter, and add ferrocyanide of potassium to clear filtrate; a precipitate will be formed in presence of albumin.

Bile pigment or acid present.—Adopt Gmelin's test (p. 138).*

Uric acid present.—Employ the Murexide test (p. 140).

Urates present.—The deposit dissolves on heating; sometimes reappears on cooling.

* More elaborate method of McNunn, Proc. Roy. Soc., No. 202, 188.

Urates dissolve in caustic alkalies; and also Uric acid is separated on adding strong acids. Apply murexide test to the deposit.

Phosphates present.—The phosphates may be in solution, or may form a deposit.

The phosphates are either in the form of amorphous phosphate of lime ($Ca_3(PO_4)_2$); crystallised phosphate of lime ($2CaH,PO_4$) or ammonio-magnesian phosphate or triple phosphate ($Mg(NH_4)PO_4 + 6H_2O$) sometimes precipitated all together, or the first and third.

If in solution, the urine is feebly acid or neutral. On boiling urine, the phosphates are deposited, the deposit being soluble in weak acid.

Deposits of phosphates are insoluble in caustic alkalies.

Why phosphates are deposited on boiling in urine which was before clear is uncertain, but possibly it may be due to heat expelling carbon dioxide, or decomposing urea into ammon. carb., which renders the urine alkaline.

Oxalates present.—The deposit is soluble in hydrochloric acid, but insoluble in acetic acid. Examine their crystalline form with a microscope.

Sugar present (Diabetes).—Sp. gr. generally high, 1,030 to 1,050.

To a small amount of urine, add caustic soda or potash, and then a few drops of copper sulphate. Shake up, and heat. The orange-coloured suboxide of copper (Cu_2O) is precipitaed. If no grape sugar be present, or too much copper sulphate be added, a black precipitate of cupric oxide (CuO) is produced (p. 134)

Quantitative estimation of grape sugar by Fehling's solution* (Trommer's test).

Diabetic urine boiled with caustic potash or soda becomes brown (Moore's test).

Estimation of sugar by fermentation.—Take sp. gr. of urine before and after fermentation. Each degree of sp. gr. lost by

* *Fehling's solution* is made as follows :—Take of—
Sulphate of copper, 40 grms.
Neutral tartrate of potash, 160 grms.
Caustic soda (sp. gr. 1.12), 750 grms.
Add distilled water to 1154.5 cc. Each 10 cc. contains ·05 grm. of sugar.

the urine represents one grain of sugar per ounce of urine (Roberts). (p. 134.)

Sugar may also be estimated by adding yeast to urine, and collecting the carbon dioxide evolved. The carbon dioxide is a measure of the amount of sugar present.

Blood present.—Examine the deposit formed on standing, with the microscope, for blood corpuscles; add to another portion a drop of tincture of guaiacum, and about a drachm of ozonic ether; a blue colour will appear at the junction of the fluids.

Pus present.—Examine deposit with the microscope.

Add caustic potash, the urine becomes stringy.

Carbolic Acid in Urine.—The urine is dark olive-green or black when first passed; on standing, a deposit resembling altered blood often takes place, and the urine becomes lighter in colour. On the addition of strong sulphuric acid, the odour of tar is exhaled from the urine. The addition of perchloride again developes a blue coloration.

Salicylic acid in urine gives a purple colour with the perchloride of iron.

Chylous Urine.—The urine may be clear or milky when passed; on standing it coagulates, forming a tremulous mass, which after a time liquefies. Examine for albumin, molecular fat, and the nematoid worm, *filaria sanguinis hominis.*

(7) ANALYSIS OF URINARY CALCULI AND DEPOSITS.

If a calculus, ignite a small portion on platinum foil. If it burn away completely, it is probably uric acid. Apply murexide test (p. 140).

Boil the powdered calculus with distilled water, or, if a urinary deposit, with the supernatant urine, and filter.

(A) INSOLUBLE PORTION.

Consists of phosphates, calcium oxalate, or uric acid.

Boil with a few drops of hydrochloric acid, and filter.

Insoluble.	*Soluble.*
Uric acid. Apply murexide test.	Phosphates or calcium oxalate. Add excess of ammonia, and then acetic acid in excess : a precipitate remaining undissolved is calcium oxalate. To the clear liquid, whether filtered or not, add ammonia : a precipitate indicates the presence of phosphates.

(B) SOLUBLE PORTION.

Urates.—Mostly deposit on cooling.

Test for urate of ammonia by boiling with potash to demonstrate the presence of ammonia, and by the murexide test for the uric acid.

Preparation of Glycogen.—*Apparatus necessary.* A solution of potassio-mercuric iodide, made by precipitating a solution of mercuric chloride with potassium iodide, washing the precipitate, and adding it to a boiling solution of potassium iodide till the latter is saturated. Any precipitate which occurs on cooling is to be filtered off. Dilute hydrochloric acid. Methylated spirit, a large bottle ; ether ; absolute alcohol.* Large funnel and Swedish filter papers. Large knife ; capsule ; several beakers ; distilled water ; ice. Mortar and pestle ; large Bunsen's burner.

Glycogen, usually obtained from the liver of animals, is also present to a considerable extent in the muscles of very young animals. To prepare glycogen, it is best to use the liver of a rabbit. The animal should be large, and it must have been well fed on a diet of grain and sugar for some days, preferably weeks, previously. The rabbit should have a full meal of grain, carrots, and sugar, about two hours before it is killed, in order that it may be in full digestion. Before destroying the animal, the capsule is to be filled with water, which is kept briskly boiling by means of the large Bunsen's burner. The rabbit is killed

* The chemicals employed in Parts I. and II. may be obtained from Messrs. Hopkins & Williams, Cross Street, Hatton Garden, W.C. ; Messrs. Burgoyne & Co., Coleman Street, E.C. ; Martindale, New Cavendish Street, W.

either by decapitation or by a blow on the head, and the abdomen is then rapidly opened, and the liver is torn out, is chopped up as quickly as possible with the knife, and is thrown into the boiling water. It is important that this operation should be performed within half a minute of the death of the animal, and that the water should not be allowed to fall below the boiling-point. The liver is to remain in the capsule for five minutes; it is then poured into a mortar, the liquid being returned to the capsule. The liver is then reduced to a pulp, and is again boiled in the capsule for ten minutes. The liquid is filtered, and the filtrate is rapidly cooled by placing the vessel in iced water. The albuminous substances in the cold filtrate are precipitated by adding potassio-mercuric iodide and dilute hydrogen chloride alternately as long as any precipitate is produced. (The albumin may also be destroyed by boiling in a strong solution of sodium sulphate.) The mixture is then stirred, is allowed to stand for five minutes, and is filtered. Alcohol is added to this second filtrate until glycogen is precipitated, which occurs after about 60% of absolute alcohol has been added. The precipitate is then filtered off, and is washed with weak spirit, strong spirit, absolute alcohol (two or three times), and finally with ether. It is then dried on a glass plate at a moderate heat, and if pure should remain as a white amorphous powder. If the water has not been completely removed, the glycogen will form a gummy mass; in this case it must be again treated with absolute alcohol.

PART III.

PRACTICAL PHYSIOLOGY.

PART III.

PRACTICAL PHYSIOLOGY.

In this division of the book, some of the chief instruments likely to be used by students in elementary physiological researches are described, and an account is given of some of the chief experiments with them. For a more elaborate account, and for a description of more complicated apparatus, reference must be made to such books as *The Handbook for the Physiological Laboratory* (Sanderson), or to Cyon's *Practisch: Physiologie.* The theoretical consideration of the experiments is to be found in the text books of physiology.

THE BLOOD.

Observation of the amœboid movements of the colourless Corpuscles.—As mentioned in Part I., it is necessary for this purpose to make use of the warm stage. Of this instrument there are various kinds in use. The simplest is a glass slide, to which a perforated circular plate of copper is cemented: this is joined to a projecting rod of the same metal. The rod communicates heat from a spirit lamp to the plate, upon which is placed the specimen of blood to be examined. The temperature is regulated by placing a small piece of cacao butter, which melts at 95° F., upon a flattened portion of the rod near the copper disc; when the butter begins to melt, the spirit lamp should be removed.

The more elaborate warm stage of Stricker consists of a metal box with a central hole. The temperature is raised either by heating a copper rod, as in the simpler apparatus, by passing a current of hot water through the stage itself, or by means of a voltaic current. A thermometer, whose reservoir encircles the central aperture, registers the temperature. If hot water be used, an india-rubber tube should be attached to the two brass

tubes which project from the sides. One of these tubes should then be placed, in connection with a jug of hot water, on a higher level than the stage of the microscope, whilst the other tube acts as a waste pipe. A syphon action may then be established, and the water will continue to circulate through the stage. To use the warm stage, place it upon the stage of the microscope, in such a position that the central aperture corresponds with the centre of the largest diaphragm, whilst the copper rod projects beyond the stage. Place a spirit lamp beneath the rod, so that it is heated; watch the effect of the heat upon the thermometer, and take care that the mercury does not rise above 39° C. When it gets near this point, move the spirit lamp further away. Whilst the stage is being heated,

Fig. 27.—Warm Stage.

prepare the specimen of blood to be examined. A drop of blood drawn from the finger, or from the tail of the newt, in the manner already described (p. 41), should be diluted with an equal bulk of normal saline solution, and should be received upon a large, square, and perfectly clean cover-glass; a second cover-glass, of the same size and shape, should also be ready. A little olive oil or melted paraffin should be evenly applied with a camel's hair brush along the edges of the cover-glass holding the blood; the second cover-glass is then carefully put over it, and the blood spreads out to form a thin uniform layer; a moist chamber is thus formed by the oil preventing evaporation. The two cover-glasses containing the blood between them are then put over the aperture in the warm stage, and examined with the highest available power. The movements of a white corpuscle being noted and recorded by drawing it at intervals of a minute.

The disadvantage of Stricker's stage is that it is expensive; a cheap modification,* which answers the purpose well, is shown in

* Made by Stanley, London Bridge.

the annexed diagram (fig. 27). It is provided with a thermometer, which registers delicately.

The gas chamber, for the purpose of examining the action of gases upon the living tissues, is a modification of the warm chamber just described. It is made by putting the tube leading from the gas generator into connection with either of the tubes which project from each side of the solid projection to which the copper rod is fixed. These tubes are on the side opposite to the thermometer; they must not be mistaken for the tubes which have been previously described as for the passage of hot water. To use the gas chamber, it is necessary to encircle the aperture with a ring of putty; the cover-glass containing the preparation to be examined is then placed upon the putty with the tissue downwards, that is to say, in the chamber. The clamp upon the gas tube is then relaxed, and the gas passes into the chamber, when its effect upon the tissue is noted. Air is re-introduced into the chamber by disconnecting the tubing, and sucking out or otherwise passing air into it.

Of counting the Blood Corpuscles.—This operation may be accomplished by means of an instrument called the *Hæmacytometer* (Gowers). (Fig. 28.)

It consists of (i) a metal tray with a central aperture and a pair of clips; the tray is larger than (ii) a glass slip which it holds. The slip is of the ordinary size, it is provided at its centre with a cell which is exactly one-fifth of a millimeter in depth, and whose floor is ruled in such a way as to form a series of squares, each measuring one-tenth of a millimeter (C). (iii) A tube (B) with a bore like that of a thermometer graduated to contain five cubic millimeters. (iv) A pipette (A) of 995 cubic millimeters capacity, with a fine aperture. (v) Elastic tubes with mouth-pieces. (vi) A small glass jar (D). (vii) A glass stirring rod in the form of a paddle (E). (viii) A guarded needle (F). (ix) Cover-glasses, wash leather, and brushes. (x) A bottle of sulphate of soda in solution of sp. gr. 1025.

Fit the elastic tubes to the pipette and to the thermometer tube, draw up by suction 995 cmm. of the sulphate of soda solution, and expel it into the glass jar. With the guarded needle, which should be perfectly clean, draw a large drop of blood from the palmar surface of the last phalanx of the left middle finger. The blood will be more easily obtained, and with less pain,

if a handkerchief has been previously wound tightly round the finger from below upwards; the blood should be drawn by a single rapid prick of the needle. Suck up the blood into the capillary tube, until it extends slightly beyond the five cubic millimeters mark; remove the excess by means of a piece of clean blotting paper applied to the end of the tube; and when exactly five cubic millimeters of blood are left, expel them into the solution of sodium sulphate which has already been measured

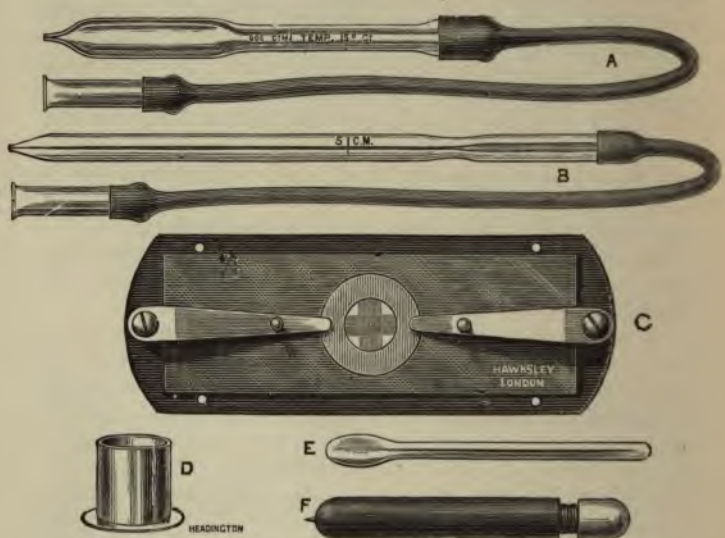

Fig. 28.—Hæmacytometer.

A. Pipette for measuring the saline solution.
B. Capillary tube for measuring the blood.
C. Cell of glass surrounding the place where the divisions are marked on the slide, mounted on a perforated metal slide with side springs to keep down the cover-glass.
D. Jar in which the blood is diluted.
E. Mixer.
F. A guarded needle.

out. Mix the blood and the sodium sulphate together by a light but rapid rotatory movement of the paddle. Place one or more drops in the cell upon the slide; cover it with a thin cover-glass, and replace it upon the tray, where it should be allowed

to remain for three minutes before the examination is commenced, in order to allow the blood corpuscles to settle. The solution of blood should exactly fill the cell, neither more nor less. In cleaning the cell, it is important that it should not be rubbed, or the micrometer lines will soon be effaced. A stream of distilled water from a wash bottle, and the subsequent use of the soft camel's hair brush, will be found to be effectual. Examine the blood with a high power (Hartnack, oc. 4, obj. 8); the corpuscles will be found lying in the squares of the micrometer. Count the number of red corpuscles in ten

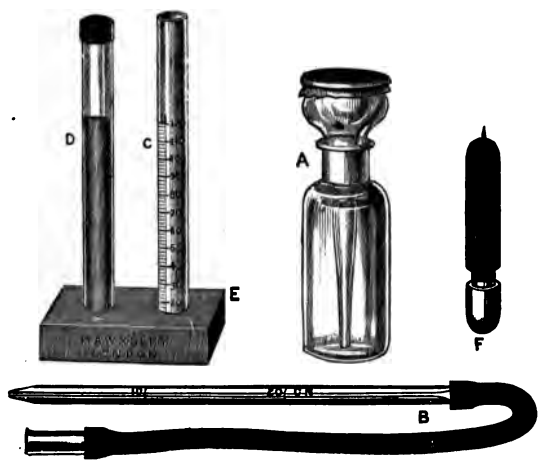

Fig. 29.—The Hæmoglobinometer (Gowers).

squares, putting down each as they are counted upon a piece of paper; add up the total and divide by ten; an average of the corpuscles for each square will thus be obtained. In the case of corpuscles which are upon the boundary lines of the squares, add or omit them according as the centre is or is not on the line bounding the square under notice at the time. In the typical healthy blood of man, each square should contain on the average fifty red corpuscles; in a woman the number is somewhat less. Examine the same ten squares, and count the number of white corpuscles; one or two should be alone present.

Estimation of the colouring matter of the Blood. *Hæmoglobinometer.**—A method of approximately estimating the amount of the hæmoglobin in blood has been devised by Dr. Gowers, with an instrument called a *hæmoglobinometer*. The theory of the apparatus consists in comparing a given sample of the blood to be examined, diluted with given quantities of water, with a standard colour solution representing the colour of a normal (1%) solution of blood. The apparatus consists of two glass tubes of exactly the same size. One contains (D) a standard of the tint of a dilution of twenty cubic mm. of blood, in two cubic centimeters of water (1 in 100), composed of glycerin jelly tinted with carmine and picrocarmin. The second tube (C) is graduated, 100 degrees being equal to two centimeters (100 times twenty cubic millimeters).

The twenty cubic millimeters of blood are measured by a capillary pipette (B) (similar to, but larger than that used for the hæmacytometer). This quantity of the blood to be tested is ejected into the bottom of the tube, a few drops of distilled water being first placed in the latter. The mixture is rapidly agitated to prevent the coagulation of the blood. Distilled water is then added drop by drop (from the pipette stopper of a bottle (A) supplied for that purpose), until the tint of the dilution is the same as that of the standard, and the amount of water which has been added (*i.e.* the degrees of dilution) indicates the amount of hæmoglobin.

Since average normal blood yields the tint of the standard at 100 degrees of dilution, the number of degrees of dilution necessary to obtain the same tint with a given specimen of blood is the percentage proportion of the hæmoglobin contained in it, compared to the normal. By ascertaining with the hæmacytometer the corpuscular richness of the blood, we are able to compare the two. A fraction, of which the numerator is the percentage of hæmoglobin, and the denominator the percentage of corpuscles, gives at once the average value per corpuscle. In using the instrument, the tint may be estimated by placing a piece of white paper behind the tubes; some light is, however, reflected from the suspended corpuscles from which the hæmoglobin has been dissolved. It will be found that during six or

* Made by Hawkesley, Oxford Street.

eight degrees of dilution it is difficult to distinguish a difference between the tint of the tubes. It is therefore necessary to note the degree at which the colour of the dilution ceases to be deeper than the standard, and also that at which it is distinctly paler. The degree midway between these two will represent the hæmoglobin percentage.

PROPERTIES OF MUSCLE.

For convenience of description the properties of muscle will be treated of in the following order :—

a. **Excitability** under stimuli.
  (1) **Electrical.**—(*a*) Action of the continuous current.
    (*b*) Of the induced current.
    (*c*) Of a series of shocks.
  (2) **Chemical.**
  (3) **Mechanical.**
  (4) **Thermal.**

b. **Elasticity.**

c. **Reaction.** (1) At rest. (2) After contraction.

d. **Transparency.**

e. **Microscopic Characters.**

(*a*) EXCITABILITY OF LIVING MUSCLE.

To show the excitability of living muscle, it is usual to employ the muscles of a pithed frog, either remaining *in situ* or removed from the body. Any muscle will answer the purpose which may be conveniently dissected out, but it is usual to employ those to which nerves can be easily traced, and to apply the stimuli to the nerve supplying the muscle, instead of to the muscle itself directly.

To pith a Frog.—In order to pith a frog, the animal must be held by its two fore-legs in such a way that its belly is pressed against the dorsal surface of the left index finger, the head projecting beyond the tip of the finger, and being pressed downwards by the thumb of the left hand. The skin on the back is then put on the stretch, and the nail of the right index finger is

drawn down the centre of the head towards the spinal column. Immediately below the head, and at its junction with the vertebræ, a slight depression will be felt, marking the position of the occipito-atlantoid membrane. A small triangular snip is made over this spot with a pair of sharp-pointed scissors, the membrane is then divided, and a wooden match sharpened at one extremity is thrust first upwards into the brain to destroy sensibility, and then downwards into the spinal canal to destroy the spinal cord. In performing this operation, no bleeding should occur. Care must be taken that the match really enters the spinal canal, and that on the one hand it does not simply pass beneath the skin of the back, whilst on the other it does not pass into the abdominal cavity. The sudden extension of the hind limbs may be taken as a proof that the operation has been properly performed.

Arrangement of the Nerve-muscle Preparation.*— Having pithed a vigorous frog, open the abdomen with a pair of sharp scissors, turn aside and remove the viscera, and expose the sacral plexus of each side; free the nerves from connective tissue, clamp the head of the frog in a holder, and attach to a retort stand, allowing the lower extremities to hang about eight or ten inches above the table. A pair of electrodes may now be inserted behind the nerves of the plexus. The electrodes are made by fixing with sealing-wax two pieces of copper wire in glass tubes about an inch and a half long, and allowing the ends to project, fastening the tubes together with sealing-wax, and soldering to one end of the projecting wires two thinner wires, two feet long, covered with cotton. The ends projecting not quite so far at the opposite end of the tubes are then scraped with a knife, and bent nearly at right angles, and the electrodes are complete. Electrodes may also be made by inserting pins two inches long into a cork, soldering wires to the heads, and turning up the points as above.

1. **Electrical Stimulation. Continuous Current.**— The nerve-muscle preparation being arranged as above, prepare a Daniell's cell. This consists of an internal zinc rod, well amalgamated, contained in a porous cell full of dilute sulphuric acid 10%; the cell is placed in the outside copper vessel, filled with a saturated solution of copper sulphate. The

* See also p. 167.

copper forms the positive pole. Attach wires to the poles of the battery, and bring them to the inner binding screws of a Du Bois Reymond's key (fig. 30). Bring the electrode wires to the outer binding screws; arrange the electrodes behind the sacral plexus of the preparation. Open the key by raising the handle, and after a few seconds close it again; a contraction of the muscles of the lower extremities will occur at the opening and at the closing, that is to say, at the make and at the break, and during the interval there will be no contraction, although the current is passing through the nerve all the time. The use of the key is to shut off the current from the nerve, as whilst it is closed the brass plate affords much less resistance to the current than the nerve; and as the current will pass in the direction of least resistance, the whole of it passes through the key to the battery again. On opening the key the current is bound to pass through the nerve, which it excites, and so produces contraction in the muscles supplied by it. The electrode connected with the copper is called the *positive electrode* or *anode*, and that connected with the zinc the *negative electrode* or *kathode*. It is as well to keep these wires distinct by having them covered with different-coloured silk (red = positive, blue = negative), or to attach to them pieces of ribbon of different colours. The current is said to pass from the positive to the negative. When the kathode is nearer the muscle than the anode, the current is said to be *descending;* and when, on the other hand, the kathode is above the anode, the current is called *ascending*. If the key be *opened* for some time, and then the battery wire be removed, a contraction will occur several times on closing the key, without the battery current; this is due to the electrodes having been polarized by the current: as many as twenty contractions may be shown under favourable circumstances in this way.

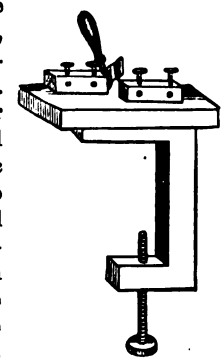

Fig. 30.
Du Bois Reymond's Key.

Single Induction Shocks.—To the two screws at the top of a Du Bois Reymond's induction coil (fig. 39, B.) which are in connection with the ends of the wire forming the primary coil,

bring the wires from a Daniell's cell, interposing a key (a Morse's telegraph key is a convenient form) to the screws at the bottom of the secondary coil, attach the electrode wires, interposing a second key. Each time the battery circuit is completed by closing the key, and broken by opening the key, an induced current is momentarily produced in the secondary coil in opposite direction on making, and in the same direction on breaking; and each of these, if strong enough, will produce a single muscular contraction. It is noticeable that when the current is strong the break shock is rather greater than the make. Open out the scale of the apparatus, and remove the secondary coil a long way

Fig. 31.—Trigger or Turn-over Key, open.

from the primary; it will be found that the current induced in the secondary coil upon opening or closing the primary circuit is insufficient to cause contraction. Move the secondary coil gradually nearer the primary, and presently there will be a faint contraction at break; note the exact point on the scale. On approaching the secondary coil nearer, a point will be reached where there will be in addition a faint contraction on make, as well as break. Next apply the electrode to the muscle itself, and compare the point at which contractions occur under these circumstances with the point at which contractions occur when the nerve itself is stimulated.

The use of the key in the secondary circuit is to cut off the make or break contraction, or both ; thus, if key 2 is open whilst key 1 is opened and shut, contractions will occur at make and break. If key 2 is open, and then key 1 is opened, a breaking contraction will occur, but the making contraction may be cut off by closing key 2 before key 1. And similarly, if key 2 is closed and then key 1 is opened, and opened before key 1 is shut, a single making contraction will occur. If key 2 is closed, whilst key 1 is opened and shut, no contraction will occur.

Series of Shocks.—*Magnetic interrupter.* Connect the wires

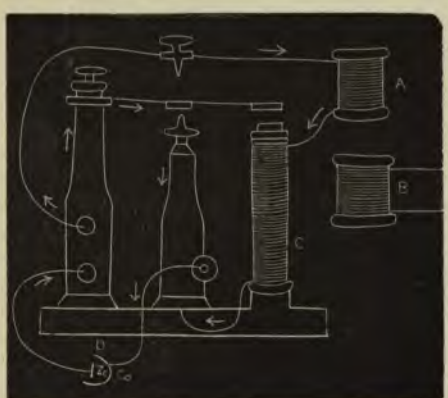

Fig. 32.—Diagram of course of currents in the magnetic interrupter (after Sanderson). The screw near the top of the fig. should be drawn so as to be in contact with the spring when the side wire is not in use. The fig. represents Helmholtz's modification.

from the battery with the screws at the bases of the two pillars of an induction coil. Interpose a key in the secondary circuit. The current passes up the outer pillar, along the spring, until it arrives at the point where contact is made by means of a platinum-pointed screw, adjustable and in connection with the end of the primary coil ; the current passes by this connection through the primary coil, and then through the coils of wire surrounding two pieces of soft iron, and thence to the battery through the middle pillar coil. As soon as the current passes

through the electro-magnet, the soft iron is magnetised, and draws down the hammer. This breaks contact with the spring, and the current is by this means interrupted, to be again made as soon as the magnet ceases to act from the stoppage of the current through the electric coil round its soft iron. In this way a series of rapid make and break shocks occur, and these are represented in the induced current, and therefore in a series of make and break contractions of the muscle, when the electrodes placed under the nerve are connected with the screws of the secondary coil, and the key is opened; the effect of this is to send the muscle into a state of tonic spasm, and the frog's leg and foot are rigidly extended. The contraction will continue for some time, but will finally give way under prolonged stimulation.

Helmholtz's modification.—This is a method by which the induced current is made to act more regularly. In the ordinary use of the magnetic interrupter, the break current is found to be much more powerful than the make; this modification equalizes make and break. It is done by connecting the outside pillar with a binding screw, which is in connection with the screw at one end of the primary coil; in this way part of the current passes at once to the primary coil, and continues to pass, whether or not the plate is attracted to the magnet. It is as well to raise the point of the middle pillar by means of the middle screw, and to remove the screw attached to the end of the primary coil some distance. The current on entering (supposing the contact between the spring and the middle pillar to be made) divides; one portion passes through the primary circuit and magnet, the other passes through the contact down the middle pillar and back to the battery. As the last circuit has but little resistance compared to the resistance of the coil, but little current passes through the coil, and so the magnet no longer holds the spring which is released. All the current now passes through the coil, the spring again descends, and so on (see fig. 32).

Extra Current.—The extra current of Faraday may be demonstrated by taking a Daniell's element, two keys, the primary coil, a nerve-muscle preparation, and electrodes. The apparatus is arranged so that both keys, as well as the induction coil, are placed in the primary circuit, whilst to the second key the electrodes are connected. On testing the current with

the tongue, supposing the key No. 1 be open, on opening key No. 2, as well as on shutting it, there is an appreciable effect upon the tongue. When the coil is cut off by closing key A, there is very little or no effect on opening key 2. The effect

Fig. 33.—Diagram of arrangement to show extra current. A, battery; B, key; C, primary coil; D, nerve-muscle preparation; the second key is not named. The wire on the left of the fig. should come from the outside copper plate, and that to the right from the zinc plate.

is produced by the extra induced current. It may also be shown by diminishing the battery current by a wire directly connecting the poles until no contraction occurs. If key 1 is closed, then, on breaking with the primary coil, a contraction will occur.

Unipolar Excitation.—Arrange the battery and coil for single induction shocks, and connect one electrode with one of the screws at the end of the secondary coil. Place this under the exposed sciatic nerve of a nerve-muscle preparation, which should be arranged on a plate of glass upon a frog board; *i.e.*, a flat piece of board covered with cork. Open and shut a key in the primary circuit, and there will be no response; now touch the muscle with the finger or a pair of forceps held in the hand, and it will contract.

Galvani's Experiment.—Take a piece of zinc, thoroughly cleaned, and coil round one end a piece of copper wire, which projects in such a way that a fork with two equal prongs is

made. Insert the zinc behind the sacral plexus of a pithed frog, and allow the copper to fall upon the thigh muscles; a

Fig. 34.—Galvani's Experiment.

contraction will occur. If the muscles be very excitable, there will also be a contraction when the copper is removed from the muscle.

Interruption by means of a Spring.—Bring one of the wires from a Daniell to the end of a steel spring which is fixed in a support in such a way that when it is set into vibration the opposite end dips into a mercury cup in metallic connection with the primary coil, and which is also connected with the battery, (the spring and cup taking the place of a key,) connect the electrodes with the secondary coil, and place them under the sciatic nerve of a pithed frog. Make the spring vibrate, and notice that if the spring is short, tetanus is at once set up, and if long, that one contraction is distinctly piled on the top of another at first, and that a more gradual tetanus is produced.

Interruption by means of a Metronome.—Insert a vibrating metronome into the primary circuit of an induction coil, and allow the series of induction shocks thus regulated to break into a nerve-muscle preparation. The limb will gradually *pass into* tetanus.

Regulation of the Strength of a Current. The Rheochord.—This is done by interposing in the circuit a graduated wire, the resistance of which is regulated. This wire is arranged to form the *rheochord* (fig. 35). The one in use is that of Du Bois Reymond. (Hdbk., p. 346.)

The instrument consists of a long box or board, on which the resistance wires are stretched. At one end are fixed several brass blocks, separated from one another, and disconnected except by fine German-silver wire. At each corner of this end are binding screws, A and B, each connected with the brass block nearest. Beginning at the block nearest A, a wire passes a considerable distance up the board, passes round a peg, and returns to the second block, from which a wire in a similar manner, considerably shorter, connects it with the next block, and so on. From the two blocks at the end of the row two thin platinum wires pass to the opposite end of the board (C), and are there insu-

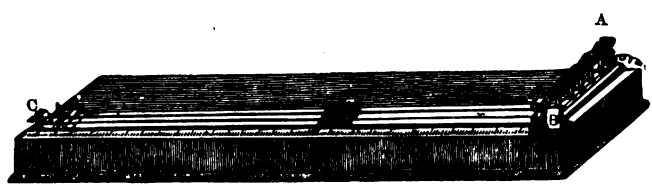

Fig. 35.—Rheochord of Du Bois Reymond.

lated; they are, however, connected by means of a slide, formed of cups of mercury. This slide is capable of being moved up and down the wires. The brass blocks are moreover capable of direct connection (except the two at the corner (B) of the board, between which are the travelling mercury cups) by the insertion of plugs. Supposing the rheochord is interposed in a continuous current in place of a key, and to the binding screws the wires of the battery are attached, as well as the electrode wires. If the slide be close to the brass blocks, and all the plugs in, the rheochord offering no resistance to the current, allows it to return to the battery, and none of it passes into the nerve. If the slide be pushed a short distance down the wires, the current meets with a certain amount of resistance, and so a fraction of the whole battery current will pass into the nerve. In like manner a greater and greater current passes into the nerve, if the slide be pushed farther away from the blocks, and if the

plugs be removed one by one, as in this way the resistance offered to the passage of the battery current through the rheochord is more and more increased.

Effect of the Strength and Direction of the Continuous Current.—Take one or two Daniell's elements, and

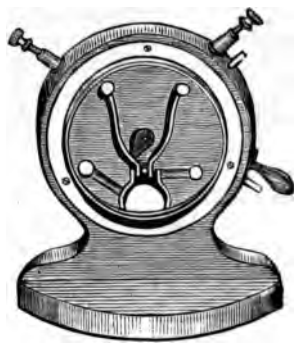

Fig. 36.—Commutator.

connect the wires from them with the rheochord binding screws, which should also be connected with a reverser or a commutator (figs. 36, 37); if the latter, to the upper screws; if the former,

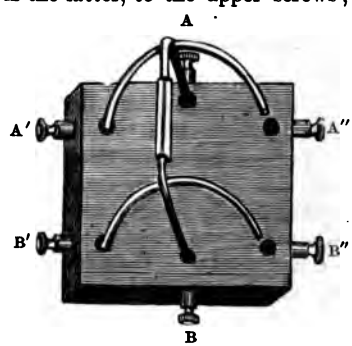

Fig. 37.—Reverser. Brass wires (not shown in fig.) connect mercury cups corresponding to A′ and B″, and A″ and B′.

to the middle screws. The electrodes are connected to the lower screws of the commutator, and to the end screws of the reverser. The handle of the commutator can be raised or lowered, and *thus* the direction of the current is changed. When horizontal,

the current is cut off. In the reverser the current is changed by rotating the arch of wire A B in the fig. to the right or left alternately.

First of all put all the plugs in the rheochord, and push the mercury cups close to the blocks. No excitation occurs on raising or depressing the handle of the commutator. Gradually increase the strength of the current, and note the point at which a contraction takes place, as well as the direction, and whether it occurs at make or break, or both. Make a table of the results, and it will be found that the make contraction of the descending current is the first to occur, then the make of the ascending. Then, as the current becomes moderately strong, make and break of both, and with a very strong current with the make of the descending and the break of the ascending.

The Rheoscopic Frog.—Prepare two nerve-muscle preparations, in the one case exposing the sciatic nerve throughout its length, and removing the skin from over the gastrocnemius only, but in the other case removing the leg with a long length of nerve. Insert the electrodes beneath the sciatic nerve of the first preparation; place this on a glass plate, in order that it may be insulated. Place the sciatic nerve of the second preparation over the thigh muscles of the first, and excite the muscles of the first with a single induced current; the muscles of the second will contract. Repeat with a series of shocks; the second preparation will be thrown into tetanus, as well as the first. Show that this is not due to escape of current by ligaturing the nerve. It is caused by the contraction of the muscles of the first producing a variation in their natural current (p. 175). This acts as a single stimulus to the nerve of the second, and so causes a contraction. Instead of passing a current through the nerve-muscle preparation, the nerve of the second may be dropped upon the muscle of the first preparation in such a way that one part of it falls upon the equator, whilst another part falls upon the muscle near its insertion into the tendon, or upon a transverse section of the muscle. The instant that the nerve falls upon these two points, the muscle of the second preparation will give a single contraction. The experiment may also be demonstrated by allowing the nerve of the limb to rest upon the exposed heart of the frog: at each systole of the heart the muscles of the limb will contract.

Effect of Urari.—Carefully destroy the brain of a frog, without allowing the escape of any blood, inject a drop of a standard solution of urari 0·1% into the posterior lymph sac, after having dissected out the sciatic nerve of one leg, and ligature the limb tightly, in order to arrest the circulation in it, excluding the nerve. Place aside under a glass shade for an hour, and test the excitability of both limbs. It will be found that the muscles of the ligatured limb will respond to stimuli applied both directly and also through the nerve, whereas the other limb will not respond to nerve stimulation, although it will to stimulation applied directly to the muscle. This experiment shows that the poison has acted upon the nerve terminations, as it has affected neither nerve nor muscle.

2. **Chemical stimulation.**—Of the same or a new nerve-muscle preparation allow the nerve to dip into a watch-glass full of strong saline solution; flickering contractions which may pass into tetanus will result. A similar experiment may be performed with glycerin. Ammonia will stimulate muscle, but not nerve; glycerin has the reverse effect.

3. **Mechanical stimulation.**—Prepare a nerve-muscle preparation: pinch or prick the nerve; contraction will occur.

4. **Thermal stimulation.**—Touch a nerve or a muscle of a nerve-muscle preparation with a hot needle; a contraction will result.

GRAPHIC REPRESENTATIONS OF THE CONTRACTIONS OF MUSCLE.

This consists in arranging the muscle-nerve preparation in such a way that on contracting the muscle raises or moves a lever, which lever is made to mark on a rapidly travelling surface. Various methods satisfy these requirements. In the first place, the muscle and nerve may be removed from the body, or may be retained *in situ*, and again the recording apparatus may be a revolving cylinder covered with blackened paper, or it may be a pendulum myograph or a spring myograph; of all of which apparatus there are many varieties.

Of the Recording Cylinder (fig. 39, A).—This apparatus consists of a cylinder or drum, which is arranged to move upon axes, revolving at definite and different rates, by means of a *clockwork* mechanism contained in a brass box, firmly resting

upon supports. The velocities of the movement are (1) slow; (2) medium fast; (3) very fast. The axes of 1 and 3 move in the same direction, viz., from right to left; 2, on the other hand, moves in the reverse direction. By means of a screw in the axis of the drum itself the drum may be raised or lowered at the will of the operator. The apparatus works as well when placed in a horizontal as in a vertical position. In the front of the box is arranged a fan in a metal frame, which regulates the clockwork movement. The movement may be stopped by means of a metal clip and handle, which are fixed so that when the handle is pressed down the clip catches the steel axis about which the fan revolves, and the clockwork is stopped. Underneath the case of the clockwork is a handle by which it is wound up. For the sake of convenience, in the front of the brass box of the clockwork are two screws, by which is fixed a metal frame, carrying a long, stout, triangular steel bar, which can be

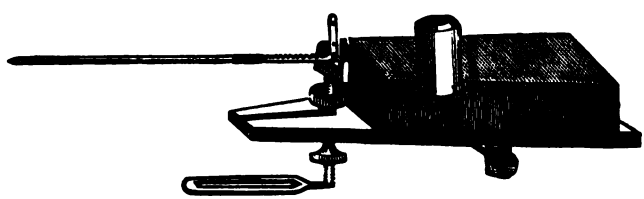

Fig. 38.—Myograph (after Sanderson).

adjusted at different positions and lengths, by means of screws in the frame. On this triangular bar, various metal uprights for carrying apparatus can be arranged. Also at one point of the lower edge of the drum is attached a metal catch for the purpose of opening a key placed in its way, called a *turn-over* or *trigger key* (fig. 39, c), the use of which is to have the muscular contraction recorded exactly at the same place on the cylinder.

Having mastered the mechanism of the apparatus, the student will then arrange it for work. The drum must be evenly covered with glazed paper, which is generally kept ready cut in strips of the size of the drum. A strip is placed round the drum, care being taken that the join should not be near the metal catch mentioned above, as in that case the tracing of the lever will be over the join, and so be spoiled. Having firmly and evenly covered the drum, it must be blackened over the smoky flame

of a small paraffin lamp or a spirit lamp, in the spirit of which camphor has been placed. For these operations the drum will have been removed from the clockwork; it may now be replaced, the clock wound up, the metal bar firmly fixed, and everything arranged in readiness for the recording operation.

Arrangement of the Nerve-muscle Preparation.—As above mentioned, the muscle and nerve may remain *in situ*, or be removed from the body. First of all, use the apparatus necessary for the former operation. This consists (fig. 38) of a triangular piece of wood, covered, to a certain extent, with cork, and with an upright cylinder of the same material fixed at the side. In front is a lever arrangement, by which the movement of the muscle in contraction is communicated to the recording apparatus. On one side is fastened a small collar, which can slide up or down an upright fixed to the triangular rod above described, and by means of a screw can be secured at any height which may be required. Pith a frog, and remove the skin from the back of one thigh; this will expose the muscles enclosed in a fine sheath of fascia; with two pairs of forceps tear the muscles apart by breaking open the sheath. Three muscles will be exposed—the *triceps* on the outside, the *semi-membranosus* on the inside, and lying between the two, and partially covered by them, a smaller muscle with tendinous ends, the *biceps*. Follow the biceps to its origin, and carefully cut it through with a pair of scissors; then catching hold of the divided end, pull it forcibly down towards its insertion, and entirely remove it. By this method of procedure the sciatic nerve will have been exposed. It may now be carefully raised, and separated from the muscle. Remove the skin from the fore-leg, and having cut through the tendon of the gastrocnemius at the *os calcis*, turn it up, and cut off the remainder of the leg just below the knee joint. Attach a strong ligature of silk or thread to the tendon, place the frog on the cork plate, firmly fix the limb by passing a long pin through the knee joint, and then attach the ligature from the tendon to the metal at right angles to the marking lever, carefully noticing that the ligature is taut, and that the muscle is really pulling on the lever; load the lever with a 10 or 20 grm. weight, and fix the myograph on the upright before spoken of, which slides along the triangular steel bar, so that the lever touches lightly the blackened surface

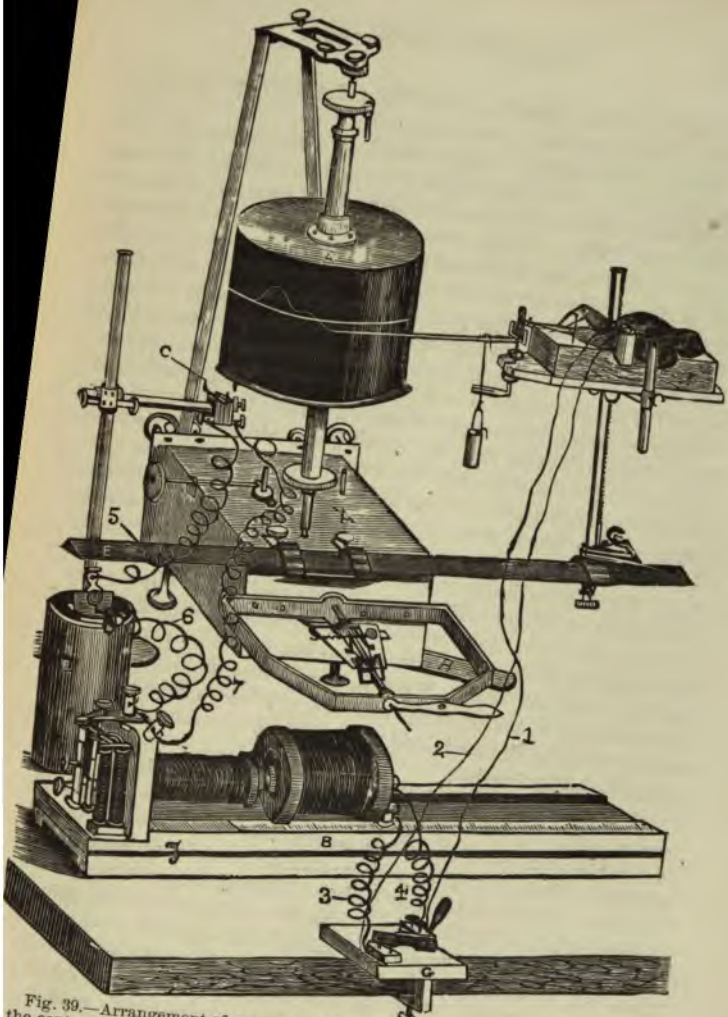

Fig. 39.—Arrangement of apparatus for recording with a revolving cylinder the contractions of muscle.

A. On the brass box containing the clockwork, the three axes are drawn near it, the cylinder is on the most anterior and most rapid axis-cylinder, also marked A.

B. Du Bois Reymond's induction coil.

C. Kick-over key in primary circuit. See also fig. 31.

D. Daniell's battery.

E. Triangular steel rod carrying the upright to which is fastened the frog-lever apparatus.

G. Du Bois Reymond's key.

1 to 7. W ires.

of the recording cylinder, with the point writing the proper way. The apparatus is now arranged.

Moist Chamber.—This consists of a moveable platform, sliding up and down the upright of a stand not unlike a medium-sized retort stand, and capable of being fixed by means of a collar and screw. The platform is made of hard wood or of vulcanite, about 3½ or 4 inches in diameter, and is furnished with two sets of binding screws for electrical purposes. These screws are continuous with wires which pass through the platform and project below, and to which battery or other wires may be attached. In the front of the platform is an opening about one inch square; upon the same upright side, (1) an electrode holder, made very simply by fixing two copper wires in a small block of wood with the centre hollowed out, the wires being stretched across the hollow, and the end fixed in the wood; they must be about one-third of an inch apart, and are to be separated by a piece of cork. This wooden block is fixed upon a holder fitted with a collar and screw to move up and down the upright. (2) A brass rod, with the circular brass holder of a screw clamp working easily in a collar at its end. The clamp holder can be fixed by a screw. A tall glass shade, large enough to cover the whole of the above, fits into a groove which runs round the platform a quarter of an inch from the edge. When in use, pieces of blotting paper, wetted with water or saline solution, are inserted beneath the shade, to keep the contained air moist; hence the apparatus is called *a moist chamber*.

Under the platform of the moist chamber is attached a metal screw collar apparatus, similarly capable of movement up and down the upright of the stand, to which is attached a fine metal spring and lever of light wood, capable of movement up and down about a fulcrum near the collar. Having prepared a nerve-muscle preparation in a manner similar to that described above, but in addition having divided the sciatic, and turned it down over the muscle, clear the femur entirely of muscle, and divide at its lower third; then fix the femur in the clamp of the moist chamber, attach a ligature to the tendon (*tendo achillis*), and carry it through the opening of the stage to the lever below, which may be weighted in the same way as in the other apparatus, with ten to twenty grams; place the nerve on the electrodes, and bring the point of the lever to write on a cylinder as before.

In the following experiments one or other of the above arrangements may be used :—

a. **Single Induced Currents.**—Arrange the induction coil, battery, and electrodes as described (p. 157), interposing the turn-over or kick-over key in the primary circuit. Cause the cylinder to revolve on the middle axis, with the key open. Before allowing the lever to touch the drum, find out the point at which the induction apparatus will give a sufficient stimulation; allow the drum to reach its proper rate of velocity; then, by means of a tangent screw, or some other delicate adjusting arrangement, make the lever touch the paper; let the catch pass the key, and then close it. In the next revolution the catch will open the primary circuit, and a contraction will occur. Mark the exact period of excitation by allowing the cylinder to make nearly another revolution, closing the key, and then with the index finger of one hand fitting the key, and with the other gradually approaching the cylinder, until the catch touches the kick-over, then slightly raising the lever, and allowing it to mark on the paper.

b. **Continuous Current.**—Proceed as in *a*; but use a Daniell's cell only, interposing a key.

c. **Faradization.**—Arrange the key in the secondary circuit, and the apparatus as usual for this purpose (p. 159). When the hammer is properly working, allow the cylinder to revolve rapidly, and open the key. A curve of tetanus will be recorded.

d. Records of tetanus curves with spring, and also with metronome, and with vibrating reed, may be taken in a manner similar to the above.

Effects of Heat and Cold.—Arrange cylinder on the second axis, allow the lever in connection with a nerve-muscle preparation to mark on the smoked paper, and set the clockwork in motion; a straight abscissa line will be drawn. Interpose the kick-over key in primary circuit, and by allowing the catch to open key, record upon this line a single muscular contraction, and mark the latent period. Now cool down the muscle by filling a test tube full of small pieces of ice, and bringing it for ten minutes into close proximity to the muscle. Then set the cylinder in motion; and when everything is in readiness, remove the test tube, and close the key. On its next revolution, the cylinder will open the key, and a muscular contraction will

occur at the same point as before; the curve will be found to be less sudden and more prolonged. By filling the test tube with water of gradually increasing temperature, a series of curves may be taken on the same line, which will show that up to a certain point the curve will be more sudden and of shorter duration.

Effects of Poisons.—Inject to $\frac{1}{10}$ mgrm. of veratria into the posterior lymph sac of a frog, and record contraction of gastrocnemius at various intervals after injection. It will be found that the full effect of this drug is to enormously lengthen the curve. Other poisons may be tried in a similar manner.

Maximal and Minimal Contractions.—Arrange a nerve-muscle preparation, and mark an abscissa line on the revolving cylinder. Find out the exact distance at which the secondary coil must be from the primary coil to give any contraction, and mark this on the abscissa line, *Minimal contraction.* Gradually increase the strength of the current, and allow the lever to write on the cylinder at distances proportionate to those at which the secondary is to the primary coil. These contractions will be recorded as ordinates, the cylinder at each contraction being motionless. It will be found that there is a point where no further increase of the strength of the current will increase the height to which the lever is raised. This is the *maximal* stimulation.

Curve of Fatigue.—After using a muscle to demonstrate a single twitch, tetanus, and for other experiments, it will be found that the contraction will after a time alter, and that when a single curve of a fatigued muscle is compared with that of a fresh one, it is found to be much more prolonged, and possibly less high, and that the latent period is longer.

Relation of the Contraction to the Load.—By loading a muscle with different weights, it will be found that with the same stimulus the contraction first of all increases* as the load is increased from zero upwards by small increments. As the load continues to be increased, the increment diminishes, and finally gives place to a decrement. The initial increase of contraction is most prominent when its stimulus lies within a certain range of intensity.

Measurement of the Time Relations of Muscular Contractions.—This is done by the vibrating tuning fork, or

* Foster, "Hdbk. Phys. Laboratory," p. 362.

by a reed made to vibrate a definite number of times in a second. The tuning fork of large size has on one prong a small style attached; and after the prong has been smartly tapped by means of a mallet or similar instrument covered with felt, the style, if applied to the revolving cylinder, will mark the number of times the fork vibrates in a second. Another way is to place the tuning fork in a battery circuit, and allow its vibrations to be communicated to a small chronograph, which writes on the recording surface. This instrument consists of a small electro-magnet. Each time the iron is magnetised it draws down a piece of metal, arranged on a frame in such a way that it can move to or from the magnet; at the other end of the frame is a small pedunculated hook, to which is fastened an elastic counterpoise. To the frame is fixed a style, capable of writing on a drum. Each time the rod vibrates, the current is made, the magnet draws down the piece of metal, and so the style makes a stroke on the smoked paper. At the break the elastic raises the style, and so on.

The Pendulum Myograph.*—In this instrument, the clockwork movement, which is frequently unreliable, is replaced by the action of gravitation. The recording plate is attached to a pendulum. On this principle several instruments differing somewhat in detail have been constructed. The pendulum with the recording plate is fixed by a catch which is capable of being moved certain distances along the arc through which the pendulum swings. When the arc is lengthened, the velocity is altered. In its swing the pendulum knocks over, with a catch attached to the middle of its lower edge, a trigger key placed in the primary circuit, and by this means the muscle of a muscle-nerve preparation gives a contraction. The preparation is arranged in a manner similar to that described above (p. 167) for the other kind of recording apparatus; but the frog apparatus is fixed in a collar to the upright of a firm stand, capable of being raised or lowered by means of a screw. The upright is fitted with a circular movement worked by a tangent screw at its base. The stand, with frog apparatus, is placed upon a firm table close to the myograph; this table can not only be raised and lowered, but is also capable of a circular horizontal movement.

* Textbook of Phys. (Foster), 3rd ed., pp. 43, 44.

Muscular Contraction with Pendulum Myograph.—Allow the pendulum to hang vertically. Arrange a nerve-muscle preparation on the table, as above described. Cover the glass plate of the pendulum smoothly with glazed paper; having smoked it, replace the plate. Adjust the lever so that it barely touches the plate at its edge; this can be done by raising the pendulum to the right of the table, and turning the table on its axis with a handle provided for the purpose, towards the left. Arrange a pair of electrodes under the nerve, interpose a key in the secondary current of an induction apparatus, and fix the trigger key (fig. 31), at a convenient place in the battery circuit. Close the key in the secondary circuit (key 2), then raise the pendulum, and fix it in the catch to the right; see to the adjustment of the writing lever. Close the trigger key, then open the second key, and set free the pendulum. As it passes the trigger key, a stimulus will be sent into the nerve by opening the battery circuit, and a contraction will be recorded on the plate as it passes the lever. Underneath this tracing, a tracing of a vibrating tuning fork must be taken, and the latent period may be marked by returning the pendulum to its place, closing the second key, and then by carefully approaching the pendulum catch to the trigger key, which can be kept closed by the finger; by slightly raising the lever, and allowing it to make a mark upon the paper, the exact point where the current entered the nerve is recorded. The effects of heat and cold, and of the action of poisons, can be demonstrated with this myograph, and also by taking two pairs of electrodes, and placing one a long distance from, and the other near, the muscle, connecting them to the end screws of a reverser (fig. 37), from which the cross wires have been removed, and alternately throwing the current into one or the other pair of electrodes; the effect of having a long or a short piece of nerve stimulated may be shown by the difference in the length of the curve. By having two trigger keys arranged at small distances, two coils, batteries, and electrodes, the effect of two stimuli acting one after the other may be shown.

The Spring Myograph.—In this instrument the clockwork is replaced by the momentum imparted by a strong coiled spring. The recording surface is, as in the case of the pendulum myograph, a glass plate; and in this instrument, as in the other, the

glass plate in its course opens a trigger key, and a contraction similarly ensues, as the apparatus, induction coil, key, and electrodes are arranged in an almost exactly similar way.

Natural Currents in Nerve and Muscle.*—*Negative variation.* Arrange a Thomson's galvanometer in a dark chamber, and place the scale at about three feet east and west facing it. Light the paraffin lamp, and, after having set the mirror free by raising the screw adjustment at the top of the instrument, adjust the lamp so that the light falls well on it. By means of the magnet, adjust the mirror until it throws its light

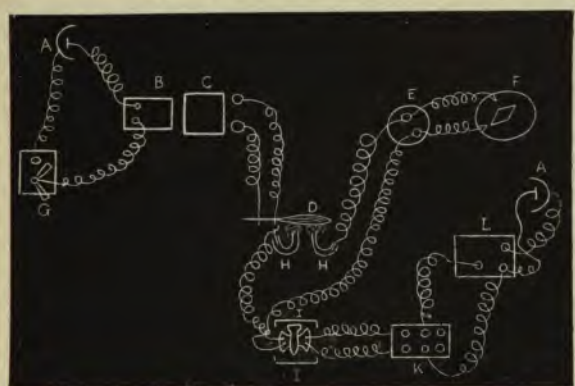

Fig. 40.—Diagram of the arrangement of apparatus for demonstrating negative variation.

A. Daniell's cell. B. C. Coil. D. Muscle nerve preparation. E. Shunt. F. Galvanometer. G. Key. H. H. Non-polarisable electrodes. I. Reverser wires connecting mercury cups not shown. K. Shunt. L. Compensator.

upon the zero of the scale. To the binding screws of the galvanometer attach the screws from the shunt, and for the sake of practice allow a weak current to pass from a Daniell; by attaching wires from the battery to the shunt, with the plug in the hole marked $\frac{1}{100}$, the needle will be deflected, as indicated by the movement of the spot of light on the scale; the light will probably move in the same direction as the current. Prepare two pairs of non-polarisable electrodes, and connect them with the shunt; the plugs being in the shunt, place the electrodes

* Treated of in Handbook, pp. 371—376.

so that their plugs touch: on opening the shunt there will be no deflection. Now take a pithed frog, and having dissected out the sciatic nerve, fix the femur in a cork plate by means of a pin, and having attached a ligature to the tendo-achillis, tie it firmly to a hook, and stretch it slightly; remove the remainder of the frog from the gastrocnemius thus prepared; place the electrodes near, having inserted the plugs in the shunt so that one touches the middle, and the other one end; now open the plug $\frac{1}{1}$, the light will quickly pass off the scale; re-insert the plug, and remove another, so as to allow less of the muscle current to pass through the galvanometer, say $\frac{1}{1000}$; then note the amount and direction of the current, from the direction of the deflection and the degrees of the scale the light has passed over. Similarly the position of the electrodes may be altered, and the currents noted, and the general law demonstrated. The scale reading of the galvanometer is proportionate to the current passing through it, "but affords no indication of the difference of potential between the two surfaces compared." For this purpose it is necessary to balance the current in the galvanometer circuit due to the electro-motive force of the muscle by an opposed current, of which the electro-motive force is known. The instrument used for this purpose is called a *compensator* (Sanderson). In the fig. 40 is represented the compensator and its standard battery. For the purpose of showing the negative variation of the muscle current when the muscle enters into contraction, under the nerve arrange a pair of electrodes connected with an induction coil. The rest of the apparatus as before.

Electrotomus, and the Law of Contraction.*—Prepare a nerve-muscle preparation, and remove it from the body, taking care that the sciatic nerve is uninjured, and very long. Place the preparation in a moist chamber, and arrange the nerve over two pairs of non-polarisable electrodes (Hdbk. p. 345); connect one pair of electrodes with an induction coil, and arrange for single shocks; connect the other pair with a rheochord, the binding screws of which are also joined to the end screws of a reverser. Find out the exact minimal current which will cause a contraction, and then move the secondary coil a little farther away from the primary to the middle screws, the wires of one

* This is treated fully in Handbook, pp. 377—388, and also "Practical *Exercises*. (Sanderson), pp. 15—18.

or more Daniell's elements being connected. On making or on breaking the primary current there will be no contraction; but if now a descending current be sent into the nerve, an induction current weaker than the normal minimal will cause a contraction. This will also occur for some time after the continuous current has been shut off. Similarly, it may be shown that if the current be ascending, an induced current, stronger than the minimal, will be required to produce a contraction; in like manner, the effects of a series of shocks may be shown, plus an ascending or descending continuous current. So we see that the irritability of the nerve is increased during the passage of a constant current in a descending direction, and diminished if it be in a contrary direction. Removing the induction coil, with the apparatus arranged as above, it may be shown that the contractions of the muscle depend upon the strength and direction of the current, by means of the rheochord and the reverse. Send a fairly powerful interrupted shock into the muscle; it will be found that there is a distinct diminution of the muscle current which occurred before faradization. The current and the negative variations in the frog's heart may be demonstrated in a similar manner.

b. **Elasticity of Muscle.**—Prepare the gastrocnemius attached to the femur, clamp the femur, attach the gastrocnemius tendon to the lever of the moist chamber, and load the lever with a ten gram weight. Allow the lever to mark on a recording cylinder, then load with twenty to thirty grams, and so on. It will be found that the extensibility gradually diminishes for equal increments of weight. On removing the weights, the lever will return to the same point on the paper from which it started.

c. **Reaction of Muscle.**—Remove from a pithed frog a gastrocnemius which has been perfectly freed from blood, cut it across with a sharp knife, and apply blue and red litmus paper to the ends. It will be found that there will be a bluish mark on the red litmus.

d. **Transparency.**—Take a flat muscle of a pithed frog (*mylohyoid* or *sartorius*), and as soon as possible place it on a slide in saline solution. Examine with a quarter inch, and focus through the muscle some vessel underneath the fibres. It will be found quite clear, and so prove the transparency of living muscle. On entering into *rigor mortis* this property disappears.

e. **Microscopic characters.** (See pp. 60, 61.)

THE CIRCULATION.

EXAMINATION OF THE CIRCULATION MICROSCOPICALLY.

THE circulation may be studied (*a*) in the web of a frog's foot. This is the most easy method, as a frog can readily be obtained at all seasons of the year; (*b*) in the tail of a tadpole; (*c*) in the caudal fin of a small fish, *e.g.*, goldfish or minnow; (*d*) in the mesentery of any of the smaller mammals, such as the mouse or young rat.

To demonstrate the circulation in the web of a frog's foot, prepare a small stand upon which to rest the body of the animal. Such a stand is readily made from a block, or by supporting a thin and flat piece of wood upon props by the side of the microscope stage. In the latter case a hole with a diameter of about three-fifths of an inch should be made near one extremity. At the end of the board nearest the hole, slits are to be cut for the passage of threads. A light-coloured frog is then to be selected, and its head wrapped in a damp cloth, whilst its body is arranged in such a manner upon the stand, that one hind-foot extends over the hole in the board, the other being tucked up out of the way. Ligatures should then be passed over the ends of two adjoining toes, and pulled tight. In this way, by a little manipulation, the threads may be fixed so as to allow of a flat surface of the web between the toes being satisfactorily examined. Care must be taken not to stretch the web to an excessive extent, lest the circulation be impeded. In the majority of cases, the frog will remain perfectly quiet for a long period of time, and this is especially the case if the nose of the animal be brought into close contact with the board upon which it lies. Occasionally the frog resists all blandishments of this nature, and exhibits the greatest restlessness. It will then be necessary to subject it to the influence of ether, or to inject beneath the skin of the back a very weak aqueous solution of urari. The effect of the drug is to render the animal motionless, by paralyzing the endings of the nerves in muscles, and thus preventing the transmission of motor impulses. Urari requires from fifteen to

thirty minutes to produce its full effect. The web, after a suitable piece has been obtained for examination, should be brought into focus, and should be examined first with a low power of the microscope (Hartnack, obj. 4, oc. 3), and afterwards with a high power (Hartnack, obj. 8, oc. 3). From time to time, during the examination, the web should be moistened with water. The examination should not be commenced until two or three minutes after the web has been fixed, in order to allow, as far as possible, the circulation to return to its normal condition.

Under the low power notice and draw (*a*) the black *pigment cells* of irregular shape and of varying size lying more superficial than (*b*) *the arterioles*, in which the blood current is more rapid than in (*c*) *the venules*, (*d*) the *capillaries*. Observe the alterations in their size. With the low power select a thin piece of the web, where the vessels can be distinctly seen, and continue the observation under the high power. Determine (*a*) the relative positions of the red and white corpuscles, the red in the centre, the white at the sides of the vessels; (*b*) the diapedesis,

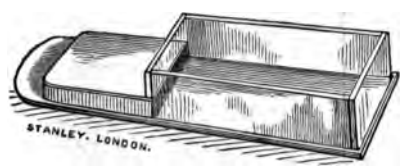

Fig. 41.—Glass for examining the circulation in the fish's tail.

or passage of white blood corpuscles through the walls of the capillaries. This phenomenon can, however, only rarely be observed.

The circulation in the tail of the tadpole is readily examined if the animal be first placed in a watch-glass full of water, to which a drop or two of urari solution has been added. When the tadpole has become motionless, it should be transferred to a slide, and examined at leisure. If it is considered necessary, the thinner portions of the tail may be covered with a cover glass. The same features will be recognized as were described in the case of the frog's web.

For the examination of the circulation in a fish, all that is required is to place the fish, generally a goldfish, in a suitable

vessel, through which a stream of water is kept running continuously. This can be done by means of Caton's trough, or with a simple glass box (fig. 41), covered over in part. Into the covered part the tail is inserted, the fish lying comfortably in the trough, which is filled with water, and into which a constant stream flows. The box is placed upon the stage of the microscope, and the tail can be now examined with a low power.

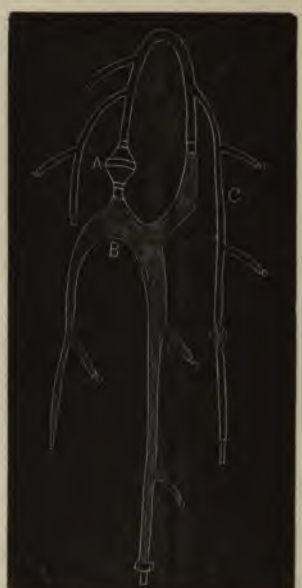

Fig. 42.—Arterial Schema.

The arterial schema (fig. 42) is an apparatus designed to represent in a diagrammatic form the main phenomena of the arterial pulse. It consists of a series of elastic tubes of varying calibre, which are arranged somewhat as they are in the vascular system, the larger tubes being equivalent to the main vessels, the smaller ones to the arterioles and venules. The ends of the tubes can be closed by means of ivory pegs, and in this way and *by clamps* the resistance within the system can be raised to

any required extent, and the varying conditions of the circulation can be imitated. In the centre of the system is a thick-walled elastic sac provided with valves, as in the case of an enema syringe, so that fluid can pass through it only in one direction. When required for use, the tubes are moderately distended with water. Three levers similar to those employed with the sphygmograph are arranged at intervals of six to eight inches along the tubes, in such a way that their movements are recorded in a vertical series of tracings upon the revolving drum. The central sac is then compressed rhythmically by the hand, and the resulting tracings made by the levers are afterwards carefully noted and compared. An explanation of the tracings thus obtained will be found in "The Handbook for the Physiological Laboratory," pp. 225, 226, plate lxxxvii., fig. 214.; and Foster's "Text-book of Physiology," ed. iii., p. 158.

The sphygmograph is an instrument used for representing graphically the characters of the pulse. In it a small button rests upon the artery, usually the radial; this button is attached to the under surface of a steel spring. The movement of the button is communicated to an upright screw, working in an arm of metal, which, with the spring, is fixed (although capable of up and down movement) by means of a screw to the frame of the apparatus. The movement of this adjustable screw is communicated to a lever of light wood, since the metal arm has in front a piece of metal projecting upwards, which comes in contact with the lever near its fulcrum. The lever writes on a smoked glass or card, which is moved by clockwork along a groove on the upper surface of the brass box containing the clockwork, which is fixed on the flat piece of metal forming the frame of the instrument. The sphygmograph is bound on to the wrist with the button on the artery (but not pressing too hard), and the clockwork backwards. The smoked surface is arranged in place; the lever adjusted by means of the screw, so that its end writes on the smoked surface by means of a sharp point. The clockwork is wound up and set going, and the character of the pulse is represented on the moving surface by means of a tracing on the paper. The clockwork is then stopped, the tracing removed, the circumstances under which it was taken noted down, and the paper or glass is varnished. Nearly all the best sphygmographs are provided with an apparatus for

adjusting the amount of the pressure which the spring exercises upon the artery, without a record of which a tracing is not of much use. The form of the instrument which is mostly used in this country is a modification of the original sphygmograph of M. Marey. The modifications * are by Sanderson, Gowers, and others. A small instrument † invented by Dr. Dudgeon is in considerable use ; it is represented in fig. 43. The advantages

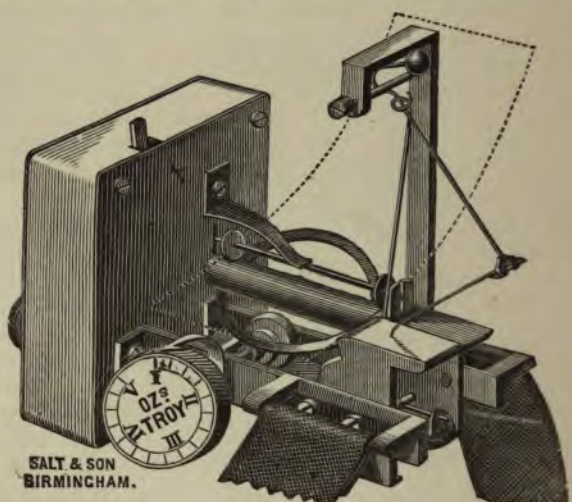

Fig. 43.– Dudgeon's Sphygmograph.

claimed for it are—It magnifies the movements of the artery in a uniform degree, viz., fifty times. The pressure of the spring can be regulated from one to five ounces. It requires no wrist-rest, and may be used with equal facility whether the patient is standing, sitting, or lying. With it a tracing of the pulse can be made almost as quickly as the pulse can be felt with the finger. Its sensitiveness is so great, that it records the slightest deviation in form or character of every beat. Its construction is so simple, that if accidentally broken, any watch-maker can repair it. It is so small (2½ by 2 inches), and it is so

* Made by Hawksley, Oxford Street.

† Made by Salt & Co., Birmingham.

light (4 oz.), that it can easily be carried in the pocket. It is only one-third of the price of the ordinary instruments.

Method for Estimating the Blood Pressure in a Rabbit.*—Make a saturated solution of chloral hydrate in ·75% saline solution; inject 15—20 minims of the chloral solution beneath the skin of the abdomen of a live rabbit. Leave the rabbit for half an hour, and in the interval prepare the rest of the apparatus. Fill a pressure bottle with a saturated solution of sodium sulphate, and suspend it by means of a string and pulley attached to the ceiling, about four feet above the operating table. The bottle should have a hole near its lower part, to which a long india-rubber tube is attached: the fluid is prevented from running out by means of a clamp attached to the lower part of the tube. Get ready Czermak's rabbit-holder, and arrange ligatures upon it for binding the fore and hind legs of the animal. Arrange the recording apparatus on one side of the rabbit-holder, and see that the clockwork is wound up, and that it is in working order. Gum a slip of glazed paper round the recording drum (p. 166), and blacken it by revolving the drum over the flame of a paraffin lamp. The coating of lampblack should be as uniform as possible, and not too thick. If the kymograph writes with a pencil or with a pen, such blackening is not requisite; in the latter case fill the can of the pen with a few drops of anilin ink. If a continuous tracing is to be taken, arrange the feeding roller in a proper position. In the case of the mercurial, or Ludwig's kymograph, see that the U tube is partially filled with clean and bright mercury; that the top of the float is not below the level of the mercury, and that the weighted thread presses upon one arm of the pen in such a way as to keep it in contact with the drum. Block up the shorter limb of the U tube with a piece of wood. Take a ⊥ shaped glass tube, connect the vertical portion with the tube descending from the pressure bottle; and one of the horizontal rami with the tube which projects from the shorter limb of the U tube, by means of a piece of india-rubber tubing (or better still, by means of a piece of substantial leaden piping). To the other horizontal ramus attach a piece of india-rubber tubing, and clamp the end; open the clamp which has hitherto closed the tube leading from the

* A license under the Vivisection Act is necessary before this experiment can be legally performed.

pressure bottle; the sodium sulphate will pass through the ⊥ shaped tube, and will fill the tubes in connection with it. Remove the piece of wood from the top of the shorter limb of the U tube, and replace it as soon as all the air is expelled, and the limb is full of sodium sulphate solution. Loosen the clip upon the india-rubber tube for an instant, and allow a few drops of the solution to escape; no air should now be present in the system of tubes thus arranged: if leakage takes place in any part, and air enters, it must be remedied. Select or make (p. 186) a cannula large enough for the carotid or femoral artery of the animal to be experimented upon; this requires considerable experience, and reference had better be made to the demonstrator. Arrange the instruments near at hand which are requisite for the operation of exposing the vessel: they are a box of scalpels, scissors, and forceps, fine and coarse, a pair of each; a pair of Krönecker's bull-dog forceps, aneurism needle, ligatures of silk, a splinter of wood (a match answers the purpose very well), a "seeker," and sponges. Fix the rabbit upon the rabbit-holder; it should be insensible at the time of the operation, and is fixed by laying it upon its back, unscrewing the bit at the end, and removing the central steel peg, pass the peg through the side of the bit, in such a way that it passes within the mouth, between the lips and behind the incisors of the two jaws, passing out on the opposite side, where it fits into a hole in the bit. The animal is thus held by the teeth. Screw up the end of the bit; the lower jaw will thus be compressed, and the animal will thereby be prevented from opening its mouth and loosening the central pin; if need be, the pressure may be increased from time to time by means of the screw. Extend the hind-legs, and pass the ligatures over the thigh on each side; tighten and fix them. Pass ligatures over the fore-legs, and fix them in such a way that the first joints are flexed, the limb being held at the elbow. Before proceeding further, see that everything is in proper working order. The points to be chiefly attended to are, (1) *The recording apparatus*, that it is wound up, that it works smoothly. (2) *The kymograph*, that the pen or style writes distinctly, and without too much pressure; that no bubbles of air exist in the tube. (3) *The system of tubes*, that they are full of the solution of the sulphate of soda, that they contain no air, that they are not kinked. (4) That *the pressure bottle* is neither too

high nor too low; and that if need be it can readily be raised or lowered to equalize the pressure of blood. (5) That the distal end of *the cannula* accurately fits, and can be easily tied into the proximal clamped end of the india-rubber tubing which is in connection with the pressure bottle and the kymograph. Expose the carotid in the rabbit. First clip away the fur over one side of the neck, and make an incision along the side of the trachea for about three inches. Separate the muscles carefully with a seeker, taking care not to go too far outwards. The artery is readily discovered; it may be distinguished from the large vein which accompanies it by its more opaque appearance, as well as its lighter colour, and by the pulsation which it exhibits. Dissect it out carefully for a short distance, and pass the aneurism needle, armed with a ligature, beneath it; withdraw the needle, leaving a loop of thread round the artery; cut the loop, and two ligatures will be thus formed. With the bull-dog forceps clamp the proximal portion—*i.e.*, the part nearest the heart—of the artery. With one of the ligatures tie the distal portion of the artery as high up as the incision will allow. Raise the portion of the artery between the clamp and the ligature by passing the splinter of wood beneath it, and with a pair of sharp and fine-pointed scissors make a V shaped incision into it. Fill the cannula, by means of a pipette, with the sodium sulphate solution, and insert it into the incision in the artery; the smaller end being directed towards the heart. Pass the second ligature over the cannula, and tighten it round the artery in such a way as to tie the cannula firmly into the vessel; (to facilitate this operation the cannula is provided with a shoulder, behind which the ligature should pass.) Fit the india-rubber tube leading through the ⊥ piece to the manometer, on to the other end of the cannula, taking care that both the tube and the cannula are quite full of the soda solution, so that no air may be enclosed at the point of junction. Remove the clamp which has hitherto prevented the escape of fluid from the india-rubber tube nearest the cannula. Set the clockwork of the recording apparatus in motion; with one hand open *cautiously and gradually* the clamp upon the tube of the pressure bottle, whilst with the other hand the bull-dog forceps are removed from the artery. If everything has been properly arranged, a few drops of blood will pass into the cannula, but the pressure

in the artery will be counteracted by the column of sodium sulphate solution, which transmits the variations in the blood pressure to the mercury in the U tube, whence it is transmitted to the lever which records it upon the drum. It is possible that the pressure bottle may be too high, and that the column of liquid is more than enough to counterbalance the blood pressure; in which case the sulphate of soda will enter the blood, and will not only vitiate the experiment, but in many cases will actually kill the animal; hence it is necessary to be cautious in opening the clamp. The sulphate of soda arrests, to a certain extent, the coagulation of the blood, and it is therefore employed; but it often happens that the cannula becomes blocked by a clot, in which case it will be necessary to detach the tubing and clean out the cannula, or it may be necessary to clamp the artery, and insert a fresh cannula.

To make a Cannula for use during Blood-pressure Experiments.—Take a piece of hard glass tubing, with a bore of about the size of an ordinary quill pen. Soften the end of the tube in the flame of a blowpipe, and draw it out gently for about an inch: there will then be a narrower portion of tube between two pieces of the full size. When the tube has cooled, heat a portion of the narrower part in the flame, and draw it out very slightly; by this means the narrower portion will be thicker in the centre than at one side. File through the middle of the narrower portion in an oblique direction. A cannula with an oblique opening at its smaller extremity will thus be formed. It must be finished by carefully rounding off its edges in an ordinary gas flame, and by filing down the aperture with a three-cornered file, until it presents the necessary obliquity. The narrowed portion will have a neck to prevent the ligature slipping off when it is tied into the artery.

The cardiograph registers in a graphic manner the heart's impulse. It consists of two portions: (*a*) A hollow metal disc whose face is covered with a thin membrane of india-rubber. The disc is provided with three levelling screws; from its posterior surface passes off a tube bent at right angles. In front of the elastic membrane is an ivory knob, which is in connection with a delicate spring arising from the side of the disc. The extremity of the spring is also provided with a pointed steel screw, resting exactly on the centre of the membrane. (*b*) The registering

portion consists of a second disc, whose elastic membrane is in connection with a lever, and from whose under surface a tube also proceeds. The tubes of the two discs are connected with each other by a portion of elastic tubing, and in this way an air-tight cavity is produced, so that any movements executed by the membrane of the first disc are reproduced in the second disc, and are transmitted to the lever by which they are recorded in the ordinary way upon the rotating drum. In using the instrument, the patient is made to lie down upon a couch of convenient height; the chest is bared, and the apex beat of the heart is found in the fifth costal interspace, somewhat below and internal to the nipple. The first disc is then applied in such a way that the ivory knob is exactly over the point at which the beat of the heart is felt. The impulse is thus transmitted to the lever, which executes certain movements. If these movements be registered upon the revolving drum, it will be found that they consist of a sudden ascent at the instant of the ventricular contraction, and of an equally marked but less sudden fall.

The Stethoscope.—The sounds of the heart are heard by means of the stethoscope invented by Laennec. This instrument is simply a cylinder of wood or metal expanded at one end into a conical portion, which is applied to the chest wall, whilst the opposite extremity is provided with a slightly concave disc, to adapt it to the observer's ear. The room should be perfectly quiet, the patient should bare his chest, and remain standing. The finger should then be placed upon the apex of the heart, which will be found on the left side in the fifth intercostal space a little below and somewhat internal to the nipple. The observer, standing in front of the patient, should apply the conical end of the stethoscope over this point, and his ear to the opposite end: at the same time he should feel with the fingers of his left hand beneath the sterno-mastoid muscle of the left side for the carotid artery. Two sounds will then be heard, one accompanying the impulse, called the *first*, or systolic sound; the other following the impulse, and known as the *second*, or diastolic sound. The first sound is the longer and more deep toned; it is best heard at the apex of the heart. The second sound is sharper and shorter; it is best heard in the third intercostal space, close to the sternum, though it is also audible at the apex. After the second sound is a pause, so that the normal cardiac cycle is

roughly represented by the rhythm *lubb, dŭp—lubb, dŭp.* In listening to the sounds of the heart, the respiratory sounds may be neglected. Care must be taken that the stethoscope be applied evenly to the chest wall, that the tube is not touched by the clothes or fingers whilst the examination is being made, and that the observer do not press so heavily against the stethoscope as to cause pain to the patient.

EXPERIMENTS WITH THE FROG'S HEART.

The Heart in the Frog.—Open the chest wall of a frog which has recently been killed by pithing, carefully remove the heart, putting it into a watch-glass containing saline solution. Examination will now show that it consists of (1) an elongated thin-walled sac, which is formed by the union of the large veins, and is the *sinus venosus;* it lies in the middle line, and on that aspect of the heart which is nearest to the spinal column. (3) Immediately in front of the sinus venosus is the *atrium*, divided internally into two auricles, and separated by a groove from (3) the *ventricle;* from the right side of the ventricle passes off (4) the *truncus arteriosus*, which quickly bifurcates into the two aortæ.

Nervous Mechanism of the Heart.—The heart removed in the preceding experiment will continue to beat for a long time after removal from the body. Snip off a piece from the sinus venosus with a pair of curved scissors; it will continue to beat: a similar result will be observed with pieces of the auricle, and of the upper part of the ventricle; *i.e.*, the portion near to the auriculo-ventricular groove. The apex of the heart, however, and the parts near it, do not beat when they are separated from the main portion of the organ.

Stannius' Experiment.—Distend the œsophagus of a recently pithed frog with a glass tube of as large a size as can be forced down it. Expose the heart, and pass a ligature round it in such a way as to constrict the junction of the sinus venosus with the atrium. The auricles and ventricle will shortly stop in diastole, *i.e.*, in a relaxed condition, whilst the sinus venosus continues to beat rhythmically. After a time the heart will recommence beating, but its rhythm will not be identical with that which it had before the application of the ligature. Whilst

the heart's action is thus arrested, prick it with a fine needle: a single beat will occur. In a similarly prepared heart separate the ventricle from the atrium, the ventricle will recommence beating, whilst the atrium remains quiescent.

Influence of the Vagus Nerve upon the Heart.—The vagus should first be leisurely dissected out in a dead frog, by the aid of the directions given in the Hdbk., p. 269, fig. 237, Pl. xcii. Repeat the dissection on a recently pithed frog, place the nerve upon a pair of electrodes in connection with an induction coil, pass a very weak current through the nerve; the rhythm of the heart will be slowed. Make the current stronger by approximating the secondary to the primary coil, and the heart's beat will be entirely and at once stopped, the cavities remaining full of blood.

Action of Drugs on the Heart.—Remove the heart from a recently pithed frog, and place it in a watch-glass with a little saline solution; the heart will continue to beat rhythmically. Drop a very dilute solution of muscarin into the saline solution; the ventricle will cease to beat. Then add to the fluid in the watch-glass a 0·2% solution of sulphate of atropia, the heart will recommence its rhythmical action.

Expose the heart in a recently pithed frog; with a delicate camel's-hair brush drop a dilute solution of the hydrochlorate of pilocarpin upon the atrium, the ventricle being at the same time moistened with a solution of atropia; the ventricle will continue to pulsate, whilst the auricles are quiescent. Antagonize the action of the pilocarpin by applying to the atrium a little atropia; the auricles will again beat.

Frog Heart and Rheoscopic Limb.—Prepare the hind leg of a vigorous frog, together with a long length of uninjured sciatic nerve, and in the same pithed frog expose the heart and open the pericardium. Arrange the frog on a glass plate, and also the limb on another, then allow the nerve to fall upon the ventricle. Each time the ventricle contracts, a contraction occurs in the limb, and if the heart is tetanized, the limb is similarly tetanized (see also p. 165).

Graphic Representation of the Ventricular Contraction.—For this purpose a hollow cylindrical box, about three inches long and one inch in diameter, is fixed upon a metal support. The box is provided with two metal tubes, by

means of which water at various temperatures may be passed through it by attaching to the metal tubes gutta-percha tubes, the one passing from a vessel fixed on a stand at some distance above the frog box, and the other similarly fixed somewhat below. The lever is thus made: a glass rod, of the thickness usually employed as a stirrer, is taken, and with a blow-pipe flame it is softened sufficiently to allow of its being drawn out at the softened part to great fineness: the fine part is then broken at a point about five inches from the unaltered glass tube, which is now similarly drawn out the other side, leaving a knob of glass between the two thinner parts. On this side all the thinned part is removed, and the glass now remains with a thin arm about five inches long. A square piece of cork is now passed along the thin glass to the knob, and through this a fine needle is passed. The needle can be adjusted in bearings which are fixed to the edge of the box. A second piece of cork is passed along the lever arm, and is adjusted and cut so that its point, directed downwards, can rest upon the ventricle of the heart. After these corks have been put in place, the writing end of the lever may be made by allowing the extremity to be softened for a few seconds in the flame of a spirit lamp. The frog heart-box can be adjusted to the recording cylinder, and for the purpose of recording the contraction the cylinder should revolve slowly. (1) Having exposed the heart of a pithed frog, tie a ligature to its frænum, and, cutting through the vessels, lift it by the ligature to the heart-box; having first allowed water at about 10° C. to pass through it, moisten the heart by placing a little serum on the top of the box by means of a capillary pipette. Adjust the lever so that the cork rests well upon the ventricle, and the writing lever marks on the recording drum record-tracings of the contractions at various temperatures, cooling the water down by means of ice to 1° C., and then raising it by increments to 20° C., and compare the tracings. (2) If, instead of a heart beating in the ordinary way, a *Stannius' preparation* (p. 188) be substituted, the heart may be stimulated by weak induction shocks, the apparatus being arranged so that the electrodes just touch the ventricle; and with a key in the secondary circuit, and the kick-over key in the primary circuit, definite regular contractions may be compared, and the effects of

temperature in altering the rapidity and the strength of the contraction seen, as well as the latent period recorded.

Endocardial Pressure in the Heart of a Frog.—A large frog (*Rana esculenta*) is taken, and the heart is exposed in the usual manner, the pericardium opened, the frænum ligatured, and the heart turned over by the ligature. A cut is made into the bulb, and by this means a double cannula is passed into the ventricle, a ligature is passed round the heart, and the cannula is tied in tightly. The vessels are then divided beyond the ligature, and the cannula, with the heart attached, is removed. To one stem of the cannula a tube is attached, communicating with a reservoir of saline solution (or dried blood * dissolved in ·75 saline solution, and filtered), which is capable of being raised or lowered in temperature by being surrounded by a metal box which contains hot, cold, or iced water. To the other end is a similar tube, which communicates by a T piece with a small mercury manometer, provided with a writing style, and also with a vessel into which the serum is received. The apparatus being arranged so that the movements of the mercury can be recorded by the float and the writing style on the slowly revolving drum, and after some serum has been allowed to pass freely through the ventricle, both tubes are clipped, the second one beyond the T piece, and the alterations in the pressure are recorded. The effects of fluids at various temperatures should similarly be recorded in the manner indicated above.

Roy's Frog-Heart Apparatus.—By this apparatus the alterations in volume which a frog's heart undergoes during contraction are recorded by the following means:—A small bell-jar, open above, but provided with a firmly fitting cork, in which is fixed a double cannula, is adjustable by a smoothly ground base upon a circular brass plate, about two to three inches in diameter. The junction is made complete by greasing the base with lard. In the plate, which is fixed to a stand adjustable on an upright, are two holes, one in the centre, a large one about one-third of an inch in diameter, to which is fixed below a brass grooved collar, about half an inch deep; the other hole is the opening into a pipe provided with a tap (stopcock). The opening provided with the collar is closed at the lower part with a membrane of animal tissue, which is loosely tied by means of

* To be had of Martindale, New Cavendish Street.

a ligature around the groove at the lower edge of the collar. To this membrane a piece of cork is fastened by sealing-wax, from which passes a wire, which can be attached to a lever, fixed on a stage below the apparatus. When using the apparatus, fix the bell-jar by means of cord, drop a little glycerin into the collar closed by membrane, and fill the jar with olive oil. Now prepare, in the way above described, the heart of a large frog, tie in the cannula, which is, as before mentioned, fixed in the cork; the tubes of the cannula communicating with the reservoir of serum on the one hand, and with a vessel to contain the serum after it has run through on the other. Pass the cannula with heart attached into the oil, and firmly secure the cork. Now open the tap, raise the membrane a little, and allow a few drops of the oil to pass out; shut the tap, and let go the membrane. By these means the lever will be found to be adjusted to a convenient elevation. Allow the lever to write on a moving drum, pass serum through at various temperatures, and compare the tracings. After a short time the heart will stop beating; but two wires are arranged, the one in the cannula, the other projecting from the plate in such a way that the heart can be moved against them by shifting the position of the bell-jar a little. The wires act as electrodes, and can be made to communicate with an induction apparatus, so that single induction shocks can be sent into the heart to produce contractions, and if need be, by means of the trigger key, at one definite point in the revolution of the recording cylinder.

Functions of the Medulla Oblongata in the Frog.—Hold the frog as if about to pith it (v. p. 155); divide the skin, occipito-atlantoid membrane and medulla by a transverse cut with a sharp scalpel; destroy the brain by thrusting a pointed piece of wood into it. The operation should be almost bloodless. The frog should be allowed to rest on its belly for a short time, to enable it to recover from the shock. Before long it will be found to have assumed a *nearly* normal attitude. It does not, however, make any spontaneous movement, provided that it is kept moist and in an equable temperature. If the flank be gently stroked, the muscles will twitch; and if the stimulus be more violent, bilateral movements will occur. This is best seen in a frog which is reely suspended. If the skin about the anus be pinched, both legs will be simultaneously drawn up.

Functions of the Roots of the Spinal Nerves.—Divide the skin along the back of a frog, whose brain alone has been destroyed, as in the previous experiment. Separate the muscles of the back, so as to expose the arches of the vertebræ, which should be carefully cut away with a pair of blunt-pointed scissors. The roots of the nerves will then be seen within the spinal canal. Expose the roots of the eighth, ninth, and tenth nerves, taking the greatest care not to touch them, by completely removing the surrounding structures. The posterior roots will then be seen to be the larger and the more superficial; they conceal the anterior roots. Select the largest of the roots now visible, it is that of the ninth nerve, and pass a fine silk ligature round it without touching it more than is necessary. Tighten the ligature. At the same instant movements will be noticed in some part of the body of the frog. Cut the nerve between the ligature and the cord; movement will again take place. Place the proximal portion of the divided nerve upon a pair of electrodes in connection with a Du Bois Reymond's induction apparatus; decided movements will occur on the passage of a current, whilst no such movements are seen when the distal extremity of the nerve is stimulated in the same manner.

Cut away the posterior root, and repeat the preceding experiments with the anterior root; movements will occur as soon as the root is touched, as well as when the ligature is tightened, and when the nerve is divided. Tetanus ensues upon stimulation of the distal portion of the nerve; but the passage of an electric current through the central end produces no result.

Functions of the Spinal Cord.—In a preparation in which the brain has been destroyed, and the cord divided below the medulla, (*a*) the reflex function of the cord may be shown by irritating the surface of the skin by means of small pieces of paper dipped in acid, and placed in various situations; contraction of certain muscles usually follows for the purpose of getting rid of the irritation. The groups of muscles are as a rule regularly brought into action when particular parts are irritated. (*b*) If the irritation occur in one leg, and that leg be prevented from moving, the other leg will act; but after a time general contraction may occur. (*c*) If the irritation be very great, or if the frog be under the influence of strychnia, general convulsions may occur.

The ophthalmoscope, brought into use by Helmholtz, consists in its simplest form of a slightly concave mirror of metal or silvered glass, perforated in the centre, and fixed into a handle; of a biconvex lens of about 2½—3 inches focal length. Two methods of examining the eye with this instrument are in common use—the direct and the indirect: the student should endeavour to accustom himself to use both methods of investigation with equal facility. A normal eye should be examined; a drop of a solution of atropia* (two grains to the ounce) should be instilled about twenty minutes before the examination is commenced; the ciliary muscle is thereby paralysed, the power of accommodation is abolished, and the pupil is dilated. This will materially facilitate the examination; but it is quite possible to observe all the details to be presently described without the use of this drug. The room being now darkened, the observer seats himself in front of the person whose eye he is about to examine, placing himself upon a somewhat higher level. A brilliant and steady light is placed close to the left ear of the patient. The atropia having been put into the right eye *only* of the patient, this eye is examined. Taking the mirror in his right hand, and looking through the central hole, the operator directs a beam of light into the eye of the patient. A red glare, known as *the reflex*, is seen; it is due to the illumination of the retina. The patient is then told to look at the little finger of the observer's right hand as he holds the mirror; to effect this the eye is rotated somewhat inwards, and at the same time the reflex changes from red to a lighter colour, owing to the reflection from the optic disc. The observer now approximates the mirror, and with it his eye to the eye of the patient, taking care to keep the light fixed upon the pupil, so as not to lose the reflex. At a certain point, which varies with different eyes, but is usually when there is an interval of about two or three inches between the observed and the observing eye, *the vessels of the retina* will become visible as lines running in different directions. Distinguish the smaller and brighter red arteries from the larger and darker coloured veins. Examine carefully the fundus of the eye *i.e.*, the red surface—until *the optic disc* is seen; trace its circular outline, and observe the small central white spot, the porus opticus, or

* Or preferably homatropin hydrobromate, as the effect passes off more rapidly.

physiological pit: near the centre is the central artery of the retina breaking up upon the disc into branches; veins also are present, and correspond roughly to the course of the arteries. Trace the vessels over the disc on to the retina. The optic disc is bounded by two delicate rings, the more external being the choroidal, whilst the more internal is the sclerotic opening. Somewhat to the outer side, and only visible after some practice, is the *fovea centralis*, with the small lighter-coloured *yellow spot* in its centre. This constitutes the direct method of examination; by it the various details of the fundus are seen as they really exist, and it is this method which should be adopted for ordinary use.

In the indirect method the patient is placed as before, and the operator holds the mirror in his right hand at a distance of twelve to eighteen inches from the patient's right eye. At the same time he rests his little finger lightly upon the temple, and holding the lens between his thumb and fore-finger, two or three inches in front of the patient's eye, directs the light through the lens into the eye. The red reflex, and subsequently the white one, having been gained, the operator slowly moves his mirror, and with it his eye, towards or away from the face of the patient, until the outline of one of the retinal vessels becomes visible, when very slight movements on the part of the operator will suffice to bring into view the details of the fundus above described, but the image will be an inverted one. The lens should be kept fixed at a distance of two to three inches, the mirror being alone moved until the disc becomes visible: should the image of the mirror, however, obscure the disc, the lens may be slightly tilted. If the observer is ametropic, *i.e.*, is myopic or hypermetropic, he will be unable to employ the direct method of examination until he has remedied his defective vision by the use of proper glasses.

The laryngoscope is an instrument employed in investigating during life the condition of the pharynx, larynx, and trachea. It consists of a large concave mirror with perforated centre, and of a smaller mirror fixed in a long handle. It is thus used: the patient is placed in a chair, a good source of light (argand burner, or lamp) is arranged on one side of, and a little above his head. The operator fixes the large mirror round his head in such a manner that he looks through the central aperture with one eye. He then seats himself opposite the patient,

and so alters the position of the mirror, which is for this purpose provided with a ball and socket joint, that a beam of light is reflected on to the lips of the patient. The patient is now directed to throw his head slightly backwards, and to open his mouth; the reflection from the mirror lights up the cavity of the mouth, and by a little alteration of the distance between the operator and the patient the point at which the greatest amount of light is reflected by the mirror—in other words, its focal length—is readily discovered. The small mirror fixed in the handle is then warmed either by holding it over the lamp or by putting it into a vessel of warm water; this is necessary to prevent the condensation of breath upon its surface. The degree of heat is regulated by applying the back of the mirror to the hand or cheek, when it should feel warm without being painful. After these preliminaries the patient is directed to put out his tongue, which is held with the left hand gently but firmly against the lower teeth, by means of a handkerchief. The warm mirror is passed to the back of the mouth, until it rests upon and slightly raises the base of the uvula, and at the same time the light is directed upon it: an inverted image of the larynx and trachea will be seen in the mirror. If the dorsum of the tongue be alone seen, the handle of the mirror must be slightly lowered until the larynx comes into view; care should be taken, however, not to move the mirror upon the uvula, as it excites retching. The observation should not be prolonged, but should rather be repeated at short intervals. The structures seen will vary somewhat according to the condition of the parts as to inspiration, expiration, phonation, etc.; they are first, and apparently at the posterior part of the *base of the tongue*, immediately below which is the arcuate outline of the *epiglottis*, with its cushion, or tubercle. Then are seen in the central line the *true vocal cords*, white and shining in their normal condition. The cords approximate (in the inverted image) posteriorly; between them is left a chink, narrow whilst a high note is being sung, wide during a deep inspiration. On each side of the true vocal cords, and apparently on a higher level, are the pink *false vocal cords*. Still more externally than the false vocal cords is the *aryteno-epiglottidean* fold, in which are situated antero-laterally upon each side three small elevations; of these the most external is the *cartilage of Wrisberg*, the intermediate is

the *cartilage of Santorini*, whilst the summit of the *arytenoid cartilage* is in front and somewhat below the preceding, being only seen during deep inspiration. The *rings of the trachea*, and even the bifurcation of the trachea itself, if the patient be directed to draw a deep breath, may be seen in the interval between the true vocal cords.

APPENDIX.

Table of Method of Examining Urine.

Colour...	...	normal, pale amber.
Smell ...	...	„ slight and characteristic.
Reaction	...	„ slightly acid.
Specific gravity		1015—1020.

Abnormal urines.

Colour—Abnormal in urines containing—

Blood (red, or smoky),
carbolic acid (black).
Bile (brown), *cystin* (yellowish green).
} Too dark

albuminous urine.
chylous „
} Too light

Excess of nitrogenous constituents (orange).
Effects of drugs, e.g., *rhubarb*, red.

Smell—*Sweet* in diabetic urine.
Very rank in urine containing excess of urea or urates.
Ammoniacal in decomposing urine.
When *cystin* is present, *sweet briar*.

Reaction—*Alkaline*, with excess of phosphates.
Strongly acid with urates in excess.

Specific gravity.
Too high in diabetes mellitus, and in excess of urea.
Too low, in ch. Bright's disease, hysteria and anæmia.

A. Containing no sediment.	B. Containing sediment on standing.	
Albumin.	Urates.	Cystin
Phosphates.	Phosphates (sometimes).	Chylous urine (becomes transparent when shaken up with ether; test for albumin).
Sugar.	Pus.	
Bile.	Mucus.	
Carbolic acid.	Oxalates.	
Blood.	Uric acid.	
Salicylic acid.	Albumin (sometimes).	
	Carbolic acid (sometimes).	

A. Abnormal Urines containing no Sediment.

Boil.

Precipitated.	Not precipitated.
Albumin.	*Sugar.*
Phosphates.	*Bile.*
Blood.	*Carbolic acid.*
	Salicylic acid.

Add nitric acid.

Precipitate dissolved.	Undissolved.
Phosphates.	*Albumin.*
	Blood.

With fresh sample of urine, apply guaiacum test (p. 144).

Blue colour.	No coloration.
Blood.	*Albumin.*

(Examine with microscope).

Apply Gmelin's test (p. 138).

Play of colours.	No play of colours.
Bile.	*Sugar.*
	Carbolic acid.
	Salicylic acid.

Apply Trommer's test (p. 143).

Reduction of copper.	No reduction.
Sugar.	*Carbolic acid.**
	*Salicylic acid.**

* *Apply special tests* (p. 144).

B. Abnormal Urines containing a Sediment.

Boil.

Precipitate or sediment dissolved.	Sediment undissolved.
Urates.	*Phosphates*—increased.
	Albumin—increased.
	Pus. *Cystin.*
	Mucus.
	Oxalates.
	Uric acid.
	Carbolic acid (see above).

Add nitric acid.

Precipitate dissolved.	Precipitate undissolved.
Phosphates (soluble in acetic acid).	*Albumin*—increased.
Oxalates (insoluble in acetic acid. Examine sediment with microscope, octahedral crystals or dumb bells).	*Pus.*
	Mucus—not increased.
Cystin (do. hexagonal plates).	*Uric acid.*

Add Caustic potash to fresh portion.

Dissolved.	Undissolved.
Uric acid.	*Albumin.*
Pus (converted into a glairy mass).	
Mucus.	
Apply confirmatory tests.	

Quantitative Estimation of Sugar in Urine.

A. By Fehling's Solution.

The urine containing the sugar is diluted ten times, and a burette is filled with the diluted urine. Ten cc. of the standard Fehling's solution is now placed in a porcelain bowl, and diluted to 1 in 10 for the sake of convenience. The bowl containing the Fehling's solution is placed on a fluid bath over a tripod, under which is a Bunsen's burner, lighted. Allow the solution to boil. It is known that 10 cc. of Fehling's solution requires ·05 grm. of sugar for complete reduction. When the solution is boiled, add to it carefully some of the urine, drop by drop, from a burette, and keep the solution stirred. Proceed in the same manner until the whole of the copper solution has been reduced. Suppose that it was found that 20 cc. of the diluted urine were required to reduce the amount of Fehling taken, or, in other words, that 20 cc. contained ·05 grm. of sugar, and 20 cc. of the original undiluted urine

contained ·5 grm. of sugar, the urine therefore contained 2·5 grms. per cent.

B. By the Specific Gravity.

Two bottles of saccharine urine are taken, and in one is put a little yeast; they are placed on the hob near the fire for 24—48 hours, or until the whole of the sugar has disappeared from the bottle in which fermentation has gone on. The specific gravity of each fluid is then taken, and each unit which the fermented urine has lost indicates (as has been found by experiment) one grain of sugar to the ounce of urine, *e.g.*, if the sp. gr. of the one be 1040, and of the other 1020, the indication is that each ounce of the fluid contained 20 grains of sugar.

The following **formulæ** may prove useful :—

Cohn's Normal Fluid.

·1 grm. Potassium phosphate.
·1 „ Magnesium sulphate (crystallised).
·01 „ Calcium triple phosphate.
20 cc. Distilled water.

And also the above with ·2 grm. ammonium tartrate added, for 'cultivation' purposes.

Pasteur's Fluid.

Potassium phosphate	20	parts.
Calcium „	2	„
Magnesium sulphate	2	„
Ammonium tartrate	100	„
Cane sugar	1,500	„
Water	8,476	„
	10,000	

Cane sugar to be omitted when Pasteur's fluid without sugar is required (Huxley & Martin).

Mayer's Solution (with Pepsin).

15 % solution sugar-candy ...	20 cc.
Dihydropotassic phosphate ...	·1 grm.
Calcic phosphate	·1 „
Magnesic sulphate	·1 „
Pepsin...	·23 „

(Huxley and Martin.)

Amalgamating Fluid, for electrodes (Sanderson).

Dissolve with gentle heat—

3 cc. Mercury in
50 cc. Nitric acid, and
150 cc. Hydrochloric acid.

Dilute with an equal volume of hydrochloric acid and eight times as much water.

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CORRIGENDA AND ADDENDA.

The reader is requested to make the following corrections of and additions to the text.

PART I,

Page 6, line 5 from top, *read* "The pieces should not be larger than the size of a hazel nut."

„ 17. Add: *Formula for alum carmine.* Boil an aqueous solution of ammonium alum with excess of carmine for ten to twenty minutes; filter, and dilute the filtrate until it contains 1 to 5% of alum. Add a few drops of carbolic acid to prevent the growth of fungus.

Formula for alcohol carmine. A teaspoonful of carmine dissolved by heating for about ten minutes in 50 cc. of 60 to 80% alcohol, to which three or four drops of hydrochloric acid have been added, and then filter.

„ 18. Anilin dyes. Class I. are soluble either in water *or in spirit.*

„ 26. Transpose "staining fluid" and "distilled water" at bottom of page, last two lines.

„ 42, line 21, for "serum" *read* "liquor sanguinis or plasma."

„ 54, line 10 from bottom, for "elastic cells" *read* "cartilage cells," and line 3 from bottom omit from "in the cartilages" to "tube."

„ 60. Add: *Unstriped muscular tissue* can be well seen in sections of the stomach of the newt or frog which have been hardened in chromic acid and stained in hæmatoxylin, and the individual cells are equally well shown in the mesentery of the newt, which has been put fresh into ammonium bichromate 5% for twenty-four hours, stained in hæmatoxylin and mounted in glycerin.

„ 62, line 11 from bottom, for "stain" *read* "stains," the endothelium covering the nerve bundles and the nodes of Ranvier.

Also add: Sometimes the nerve fibres strongly resist the action of hæmatoxylin and carmine. When this is the case, it is best to place them in ether or chloroform for twelve hours; they must then be washed in alcohol, and left in a solution of the staining re-agent for twenty-four to forty-eight hours.

Page 63, line 11, omit paragraph "It probably . . . membrane."

„ 64. Add, line 10 : Preparations of the large multipolar ganglion cells may be made from stained sections of the anterior cornua by careful teazing under a dissecting microscope.

„ 66. Ink is a very good stain for the cells of Purkinje.

„ 70, line 9, after "muscular" insert "coat." And add : Capillaries may be well shown in the mesentery of a frog which has been injected with ¼% of silver nitrate.

„ 71, last line, for "ides" *read* "sides."

„ 75, line 27 (crescents of Giannuzzi).

„ 77, line 19, after "anilin" insert hyphen.

„ 79, middle of page, for "absoprtion" *read* "absorption."
Note.—Part I. instead of Part III.

„ 80, line 15, for "submucuous" *read* "submucous."

„ 82. For "radicals" *read* "radicles" ; line 13, $\frac{1}{500}$.

„ 86, line 7 from bottom, after "circular" insert "or."

„ 87, line 15, omit "of."

„ 94, bottom line, for "it" *read* "them."

„ 95, line 5 from bottom, add after "glands," "and notice."

„ 96, line 18, for "iliac" *read* "external iliac."

„ 97, line 1, for "cavernosum" *read* "spongiosum."

„ 98, line 5 from bottom, after (2) insert A.

„ 104, line 15, omit "with."

„ 109. Add : The eyelids and the lachrymal apparatus may be obtained from a pig if human material is not available. They should be placed in the usual solution of chromic acid and spirit.

PART II.

„ 122. 1st Proteid reaction : after "ammonia" add "or potash."
3rd „ „ The soda should be added first. The colour is deepened on heating.
4th. The acetic acid in excess, and the potassium ferrocyanide in slight amount.
All act on polarised light, turning the plane of the polarised ray to the left.

„ 124, line 7. The tannic acid must be in strong solution to cause precipitation.
In next paragraph, "by" should be inserted before lead acetate and each of the other re-agents.
After description of Heller's test insert (*white or yellow*).

Serum albumin is *not* easily precipitated with strong hydrochloric acid, egg albumin is.

Page 126. The acid albumin has been formed during the precipitation of casein. Line 5 from bottom, for "lable" *read* "label."

„ 127, footnote, invert * and †.

„ 129. line 10, insert "in" before "ether."

„ „ line 11, "absolute" before "alcohol."

„ 130, line 3, for "100" *read* "1000."

„ 132, line 10 from bottom, omit "from ox gall."

„ 133, line 2, end, add "and caustic potash."

„ 134. Transpose lines 7 and 8 from bottom.

„ 139, line 3 from bottom, add "when urine is concentrated."

„ „ line 4 from bottom, for "the" *read* "that."

„ 140, line 4, for "is" *read* "are."

„ 141. line 9 from bottom, for "bicarbonate" *read* "carbonate."

„ 142, foot-note, for "McNunn" *read* "McMunn."

„ 143, last line, for "contains" *read* "is reduced by."

„ 144, line 18 from top, for "again" *read* "of iron."

PART III.

„ 157. After positive pole, add, "although the negative plate. At the negative plate is accumulated the positive electricity."

„ 159. Explanation of fig. 32. The wire at the left of the figure should be in connection with the copper, and that at the right from the zinc.

„ 161 and 175, figs. 33 and 40, add "after F. J. M. Page."

„ 162, line 1, after "plexus," add "or lumbar nerves."

„ 174, line 13 from bottom, before "one" *read* "upon the nerve."

„ 176. Heading, for "Electrotomus" *read* "Electotonus."

„ 186. After ("b") add "Marey's Tambour."

„ 189, line 6 from bottom, omit "and if . . . tetanized."

„ 192, line 2 from bottom, for "reely" *read* "freely."

„ 195. Transpose *yellow spot* and *fovea centralis*, and *read* "is the yellow spot with the small lighter-coloured fovea centralis."

APPENDIX.

„ 200, last line but one, *read* "that 20 cc. of the diluted urine contained ·05 grms. of sugar, then 20 cc. of the original undiluted urine would contain ·5 grms. of sugar, which is equivalent to 2·5 grms. per cent. of sugar.